Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD)

Progress Reports

January 2008

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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 science 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. Utilizing several sites with samples of varying ages and demographics and novel animal models, we will continue to establish procedures for better defining and characterizing the range of outcomes from prenatal alcohol exposure. These procedures are based on a multidisciplinary approach, including both basic and clinical projects for this study of dysmorphia, neuropsychological and behavioral functioning, and brain imaging. Within this aim, new, innovative techniques will be assessed and then implemented as indicated by the data. These innovative techniques will help in defining characteristics indicative of prenatal alcohol exposure that can be assessed easily and reliably.
2. Our consortium builds upon existing research programs within the current CIFASD and new sites, to build a subject population large enough to adequately address many of the questions posed in this application. Some countries have much higher rates and levels of alcohol consumption during pregnancy and FASD than does the United States and we will utilize these resources. Our goal is to obtain concordance on a set of diagnostic criteria between sites, basing these criteria on the diagnostic techniques established in Aim #1.
3. The Basic Science Component seeks to uncover mechanisms involved in alcohol teratogenesis and to design effective interventions to mitigate or prevent the effects of prenatal alcohol exposure. The basic science projects use models ranging from molecular analysis of alcohol antagonist actions to prenatal alcohol effects in mice, rats and sheep. Basic science projects will be integrated throughout by a common focus on neural systems that are vulnerable to ethanol in humans, new diagnostic procedures and effects of agents that prevent ethanol teratogenesis. The Basic Science and Clinical Components will work collaboratively to translate fundamental scientific observations into clinically useful diagnostic tools and treatments.

The Primary Goals of the Administrative Core are:

1. Provide scientific and administrative direction, leadership and oversight to the CIFASD. The PI will ensure that CIFASD investigators adhere to its goals and mission, as well as assist and coordinate interactions between various projects. It also assists and provides necessary administrative support to the Science Advisory Board and the two Steering Committees and functions as the main liaison to NIAAA regarding the CIFASD.
2. Facilitate communication between the various projects using the CIFASD web site, scheduled monthly conference calls, the biannual meetings of the Steering Committees, and the preparation and distribution of annual progress reports. It also serves a liaison role for the Administrative Core, the Steering Committees, and the Science Advisory Board, facilitating the interaction and communication of consortium scientists.
3. Monitor the cores and individual research projects for progress.
4. Provide assistance where necessary with data collection and ensure that data from the projects are uploaded into the Central Repository of the Informatics Core in a timely fashion. It will work with the Informatics Core to develop online interactive capacity among CIFASD investigators, and make most of these databases available to outside, interested scientists.
5. It will assist the Science Advisory Board and the Steering Committees in their annual evaluations of progress for each of the projects and the two major components. In conjunction with the Steering

Committees, it will establish annual priorities and deal with issues related to allocation of resources. It will also provide a mechanism for the evaluation of the projects.

6. Administer the Exploratory/Developmental Projects of the CIFASD and oversee the administration of the Exploratory/Developmental Projects.
7. Facilitate the recruitment of new scientists and new technologies into the consortium.

IV. Methods:

1. Regular monthly conference phone calls are conducted through the use of AccuConference. The Basic Science and Clinical Research Components each have their own monthly call. Area groups conference calls initiated.
2. Website maintained.
3. Biannual Meeting and Progress Reports.
4. Interaction with and help to facilitate goals of Central Repository/Informatics Core.
5. Annual Evaluations/Priorities.
6. Exploratory/Developmental Projects: Two developmental projects are included in the Administrative Core.

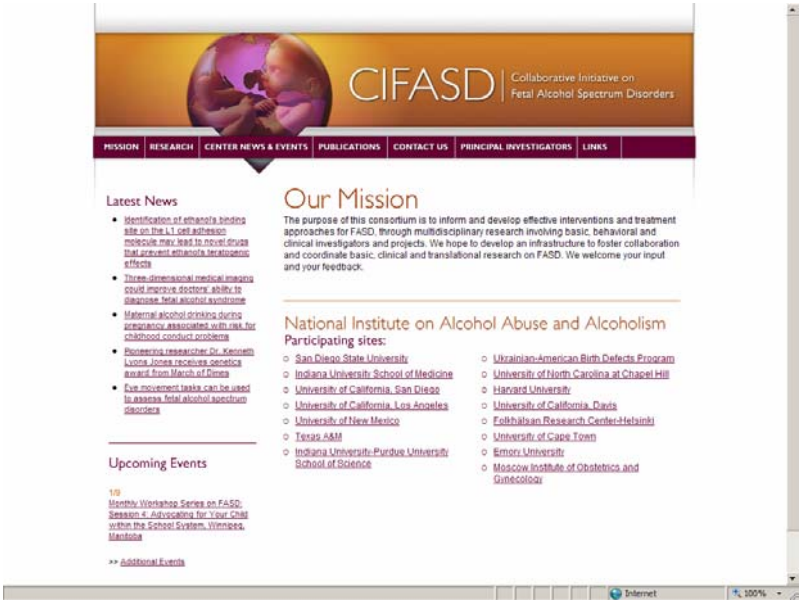
V. Accomplishments and Results:

1. Perhaps the major accomplishment of the Administrative Core since the last Progress Report was the successful direction and oversight of the competitive renewal for the CIFASD. Anticipating that an RFA for new applications for a CIFASD might be coming from NIAAA, the Administrative Core and the Steering Committee began regular discussions of a renewal. These discussions focused on reformulating the CIFASD with better integration of projects, increasing the basic science projects, evaluating existing projects, soliciting new proposals, and identifying any new aims and goals for the CIFASD. The Advisory Board also played a role in this discussion at the last biannual meeting. We announced the possibility of a competitive application process through various channels and personal invitations. Interested researchers were asked to submit a 5 page application that was reviewed by members of the Advisory Board and outside consultants solicited by the Administrative Core. Eighteen applications were ranked, much like a normal NIH application, and discussed in a telephone conference call with NIAAA staff, the Scientific Director, the PI of the Administrative Core, and Dr. West, who chaired the review. The following applications were selected for inclusion based upon their scientific ranking and previous collaborations with CIFASD investigators, since the ability to work together is imperative to the success of the consortium.

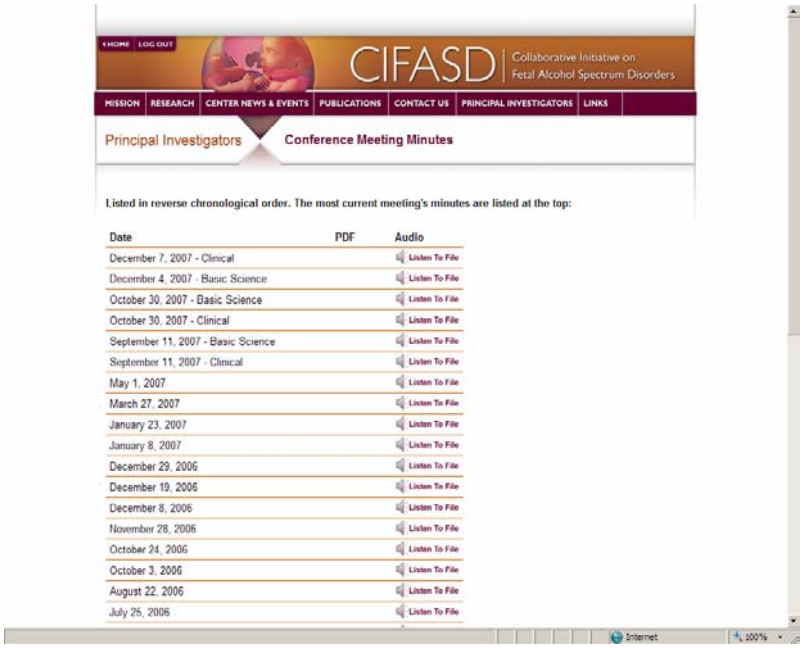
CORES	PI	APPLICANT ORGANIZATION
CIFASD: Administrative Core	Riley, Edward P.	San Diego State University
Dysmorphology Core	Jones, Kenneth Lyons	University of California, San Diego
Informatics Core	Stewart, Craig	Indiana University
CLINICAL COMPONENTS U01		
Spectrum of and Nutritional Risk Factors for FASD in Russia and Ukraine	Chambers, Christina D.	University of California, San Diego
3D Facial Imaging in FASD	Foroud, Tatiana	Indiana University School of Medicine
A Multisite Neurobehavioral Assessment of FASD	Mattson, Sarah N.	San Diego State University
School Age and Early Interventions for Children with FAS/FASD	May, Philip A.	The University of New Mexico
Mapping the Brain, the Face and Neurocognitive Function in FASD	Sowell, Elizabeth	University of California, Los Angeles
BASIC SCIENCE COMPONENTS U01		
Translational Studies of FASD using a Sheep Model	Cudd, Timothy	Texas A & M University
Molecular Pathogenesis of Fetal Alcohol Spectrum Disorders	Miller, Keith	Massachusetts General Hospital, Boston

Magnetic Resonance and Diffusion Tensor Imaging of a Mouse FASD Model	Sulik, Kathy	University of North Carolina, Chapel Hill
Mouse Model Neuro-Facial Dysmorphology: Translational & Treatment Studies	Zhou, Feng	Indiana University School of Medicine
DEVELOPMENTAL PROJECTS – Part of Administrative Core		
Prenatal Ultrasound and the Early Detection of FASD	Hull, Andrew D.	University of California, San Diego
Choline Availability and FASD	Thomas, Jennifer	San Diego State University

2. The Administrative Core coordinated the submission of these projects, and wrote the common sections for each application that were required by the RFA (e.g. Background & Significance, Progress, Data Sharing).
3. The Administrative Core oversaw and coordinated the reverse site visit held in Bethesda on May 22, 2007, including arranging for practice sessions.
4. Of the 12 U24 or U01 projects, 10 were funded and priority scores overall were very good. Eight projects had scores of less than 161.
5. The Administrative Core added new members (Calhoun, Espy, Savage) to the Science Advisory Board.
6. The Administrative Core continues to maintain and update the website. Latest news is updated on a regular basis, as are upcoming events.



7. Regular Basic Science and Clinical Research Component monthly conference phone calls have been convened. These conference calls are available verbatim on the CIFASD website. Listservs are in place in cooperation with the Informatics Core.
8. The Administrative Core assists in facilitating special area group conference calls.
9. The Core arranged the biannual meetings.
10. New investigators were added to the CIFASD. Kathy Sulik and Tim Cudd have new projects in the CIFASD. In addition, Peter Hepper of Scotland has been funded to do a small ultrasound project, and Peter Martin of Australia is doing a small epigenetic study.



11. CIFASD forms, manuals, etc, have been made available to the international research community.

12. CIFASD Community/Scientific Outreach: The PI has given several talks about the Consortium. Publications are being posted on CIFASD.org, which is updated monthly.

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 exploratory projects and requesting no cost extensions on existing 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 ensure that the both the Basic and Clinical Research Component's needs are met to execute their research goals and to encourage and help coordinate collaborations between these research components.

IX. Publications:

Not applicable.

X. Posters and Presentations:

International Research on Fetal Alcohol Spectrum Disorders, 2nd International Conference on Fetal Alcohol Spectrum Disorder, Research, Policy and Practice Around the World, March 2007, Victoria BC.

Brain and Behavioral Changes Following Prenatal Alcohol Exposure, 2nd International Conference on Fetal Alcohol Spectrum Disorder, Research, Policy and Practice Around the World, March 2007, Victoria BC.

The Foetal Brain & Alcohol – Defining Foetal Alcohol Spectrum Disorder (FASD), Consequences for Children Affected by Maternal Drug & Alcohol Usage, Parents for Children, Sheffied, England, May, 2007

"FASD – Not Just Another Pretty Face: Effects of Prenatal Alcohol on Brain & Behavior," Mac Keith Meetings, Royal Society of Medicine, London, UK, November 2006.

"New Directions in FASD Research: Where Should Scientists be Going?", The STARFISH conference, Lubbock, TX, July 2006.

"Where Are We and Where Are We Going in FAS Research?" University of Oklahoma Health Sciences Center in St. Petersburg, Russia, June, 2006.

Progress Report for the Informatics Core

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

II. Title of Project: Informatics Core

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 software tools that facilitate the accomplishment of the scientific mission of CIFASD.

IV. Methods:

CIFASD is collecting several different types of data: Dysmorphology, Neurobehavior, 2D facial images, 3D facial images, Alcohol and Control, Ultrasound, Infant Neurobehavior, and Brain images. 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 are 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. Data dictionaries are critical aspects of information repositories as they ensure the validity of data categories and provide a mechanism by which data from different projects may be compared. 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 in January, 2006.

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

	Input Tool	
	Central Repository Schema	**Updated** *
	Upload Tool	*In progress* *
	Report Tool	*In progress* *
Neurobehavior	Data Dictionary	**Updated** *
	MS Access Input Tool	**Updated** *
	Central Repository Schema	**Updated** *
	Upload Tool	**Updated** *
	Report Tool	**Updated** *
Neurobehavior, phase II	Data Dictionary	*In progress* *
	MS Access Input Tool	*In progress* *
	Central Repository Schema	*In progress* *
	Upload Tool	*In progress* *
	Report Tool	*In progress* *
3D Facial Images	Data Dictionary	**Finished**
	Central Repository Schema	**Updated** *
	Upload Tool	**Updated** *
	Report Tool	**Updated** *
2D Facial Images	Data Dictionary	
	Central Repository Schema	
	Upload Tool	
	Report Tool	
Ultrasound	Data Dictionary	**Updated** *
	MS Access Input Tool	**Finished** *
	Central Repository Schema	
	Upload Tool	
	Report Tool	
UCSD Screener	Data Dictionary	**Finished**
	MS Access Input Tool	**Finished** *
UCSD Follow-up/Outcome Interview	Data Dictionary	**Finished**
	MS Access Input Tool	**Finished** *
Infant Neurobehavior [Bayley & Maternal Questionnaire]	Data Dictionary	**Finished** *
	MS Access Input Tool	**Finished** *
	Central Repository Schema	
	Upload Tool	
	Report Tool	
Brain Imaging	Data Dictionary	
	Central Repository Schema	
	Upload Tool	
	Report Tool	

Consortium researchers may download MS Access input tools from our existing web page and soon from our new web page at https://cifasd.uits.iu.edu:/downloads/. Example screen images of data entry and retrieval tools we have created are shown in Figures 1 and 2.

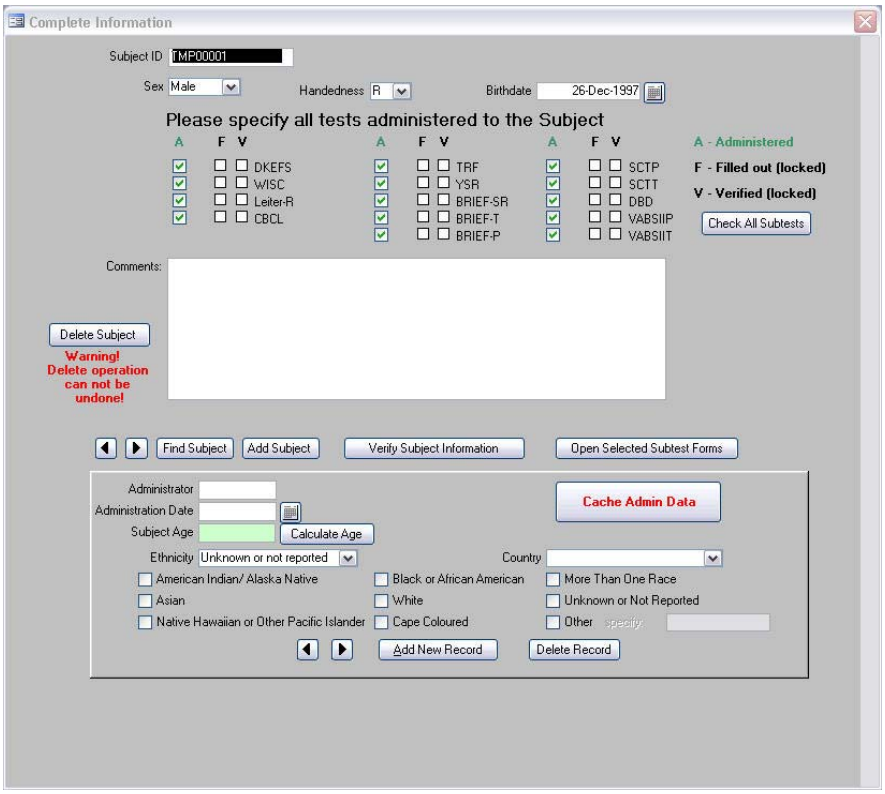


Figure 1: Neurobehavior Phase II Data Entry Input Tool. This data entry form includes double-entry data validation functionality to decrease data entry errors as well as range checking that makes it impossible to enter values outside the permissible ranges identified in the data dictionary.

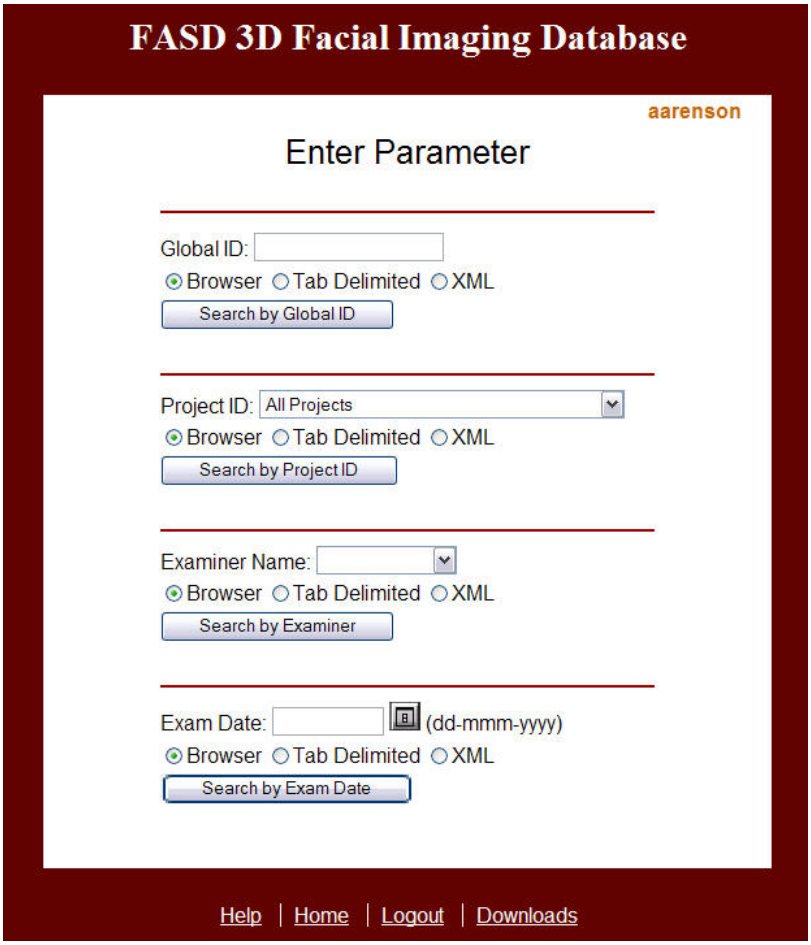


Figure 2: Web-based 3D facial imaging data access and retrieval tool.

V. Accomplishments and Results (continued):

Data for the consortium are stored in duplicate – one copy in a robotic tape storage system in Indianapolis, IN and a second copy in a robotic tape storage system in Bloomington, IN. This ensures that the consortium's valuable data will be kept reliably even in the event of a disaster at one of Indiana University's two computer rooms.

VI. Discussion:

The period from January, 2006 through the end of the previous project in September, 2007 primarily involved work extending and refining existing software rather than creating new software, though there were a few exceptions. The status table above, therefore, shows many statuses as 'Updated', reflecting the further work that was done to add new variables or functionality in response to changing project needs. Examples of new functionality include the addition of variables representing recruitment groups, extension of the Dysmorphology Input Tool to require double-entry verification, and the inclusion of dynamic encryption when uploading 3D Facial Images. There were also many instances of needing to respond to one time requests for data curation, such as fixing the global identifiers for some records or helping a project to transform data into a form that could be submitted to the CIFASD Central Repository.

One of the unexpected tasks that cropped up many times was the alteration of allowable ranges in the Input and Upload tools as researchers became more familiar with the instruments being used to collect variables. Time for such tasks was factored into estimates of required resources for the renewal.

Since the new grant began in September, 2007, a concerted effort has gone into rapidly creating new software tools. The Informatics Core worked with the Administrative Core and the various projects to navigate the conflicting priorities across the consortium as many projects were understandably interested in getting software tools quickly. The first tools to be created were the Neurobehavior phase II Input Tool and the Infant Neurobehavior Input Tool sections for the Bayley and Maternal Questionnaire variables.

The creation of the Bayley section of the Infant Neurobehavior Input Tool was made somewhat easier by the ability to make use of an existing MS Access form. The benefit of reusing the existing Bayley form, though real, was modest compared to the overall effort, contributing approximately a 10-15% reduction in effort.

The Neurobehavior phase II data dictionary and Input Tools gained very significant cost savings by being able to build from similar variables and functionality in the original Neurobehavior data set. Of the fifteen tests included in the phase II battery, five of these required no change from the original battery, two required small to moderate changes, one required significant changes, and one, CANTAB, has not been analyzed yet but will probably be able to be reused to at least some extent.

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

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

1. The upload and query tools for the phase II Neurobehavior variables are the next software tools to be created. These will be crucial for allowing integration of data from across the consortium to occur in time to for the reporting of results for the first grant year.
2. Other software to be developed in the next six months include an expanded mechanism to enable the 3D Facial Imaging Project to securely share and store data and an extension to the Ultrasound data dictionary and input tools to include biophysical profiling variables.
3. The Informatics Core will continue to work with the entire consortium to understand the ongoing needs for software tools and to set priorities for tool development.
4. The Informatics Core is continuing preparations for a paper that will allow the data dictionaries developed by this consortium to be published.

IX. Publications:

Arenson, A. D., Bakhireva, L., Chambers, T., Deximo, C., Foroud, T., Jacobson, J., Jacobson, S., Jones, K. L., Mattson, S., May, P., Moore, E., Ogle, K., Riley, E., Robinson, L., Rogers, J., Streissguth, A., Tavares, M., Urbanski, J., Yezerets, H., & Stewart, C.A. (2007). Implementation of a distributed architecture for managing collection and dissemination of data for fetal alcohol spectrum disorders research. *GCCB 2006, LNBI 4360*, 33-44.

X. Posters and Presentations:

Not applicable.

Progress Report for the Dysmorphology Core

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

II. Title of Project: Dysmorphology Core U24

III. Objectives:

1. To assure consistency as well as accuracy in recognition of Fetal Alcohol Spectrum Disorder (FASD) at all CIFASD project sites where children as being evaluated.
2. To delineate the full range of anomalies in children prenatally exposed to alcohol in order to determine the boundaries that encompass FASD in prospective as well as retrospective studies involved in the Consortium.
3. To identify specific structural features or clusters of features that are predictive of or correlated with deficits in neurobehavioral development across developmental ages spanning from infancy to adolescence.
4. To correlate the specific structural features or clusters of features identified on the CIFASD standard physical examination with alternative or complimentary diagnostic methods that are being tested in other CIFASD projects.
5. To better understand the extent to which structural features of FASD are related to specific defects in development of the brain.

IV. Methods:

1. To perform careful physical examinations, blinded to prenatal alcohol exposure, of each child ascertained at all consortium sites and to correlate the incidence of features noted on those physical exams with specific doses, gestational timing and patterns of prenatal alcohol exposure.
2. To compare the dose/timing/pattern of findings between humans and animal models from the basic science cluster studies proposed by F. Zhou (mouse), T. Cudd (sheep), and K. Sulik (mouse).
3. To correlate findings on the CIFASD standard physical exam with neurobehavioral impairment in various domains as measured in both retrospective and prospective studies.
4. To compare the predictive value of specific features of clusters of features in prospectively ascertained vs. retrospectively ascertained subjects as well as in infancy versus childhood.
5. Associate the predictive value of specific features or clusters of features with the dose/timing/pattern of prenatal alcohol exposure and compare these findings with those in the animal models from the basic science cluster studies.
6. Correlate the specific feature or clusters of features identified on the CIFASD physical exam with those identified by the 3D facial images of the same children through the 3D Facial Imaging Clinical Research Project.
7. Correlate the specific structural features or clusters of features identified on the CIFASD physical exam with those identified by prenatal ultrasound project.
8. Correlate the specific structural features identified on the standard physical exam with abnormalities identified on through the Neuro-imaging Clinical cluster project.
9. Compare the correlation of the physical findings with the neurobehavioral findings in humans with comparable neuro-imaging findings in the basic science cluster mouse project.

V. Accomplishments and Results:

Objective 1: To assure consistency as well as accuracy in recognition of Fetal Alcohol Spectrum Disorder (FASD) at all CIFASD project sites where children are being evaluated.

Total physical exams performed since the beginning of the new grant period: San Diego – 9 cases

Objective 2: To delineate the full range of anomalies in children prenatally exposed to alcohol in order to determine the boundaries that encompass FASD in prospective as well as retrospective studies involved in the Consortium. Accomplishment of this objective will be possible only after correlation has been made between physical features, neurobehavioral testing and the dose/timing of alcohol exposure, and pattern of prenatal alcohol exposure. Because the alcohol exposure data is much more accurate in the prospectively ascertained cases, this objective will be more successfully accomplished as more cases are completed in the prospective studies in Russia and Ukraine.

Objective 3: To identify specific structural features or clusters of features that are predictive of or correlated with deficits in neurobehavioral development across developmental ages spanning from infancy to adolescence. This has been a major focus of our efforts. In collaboration with Drs. Tina Chambers, Sarah Mattson, and Claire Coles we are using retrospective data from South Africa, Finland, the Great Plains, San Diego, and Moscow to document the predictive value of specific alcohol-related structural features for IQ measures by child's age category.

Objective 4: To correlate the specific structural features or clusters of features identified on the CIFASD standard physical examination with alternative or complimentary diagnostic methods that are being tested in other CIFASD projects. Analysis of the 3-D facial imaging data has been used to identify the measurements that most efficiently differentiate alcohol exposed from control subjects. As is indicated in the 3-D Facial Imaging Progress Report, 276 subjects were recruited from four sites (South Africa, Finland, Buffalo, and San Diego). Computerized anthropometry was employed to identify facial features that could distinguish FAS patients from controls across a wide age range and across ethnically disparate study populations. Subjects were placed into one of four populations based on their ancestry (Cape Coloured, Finnish Caucasian, African American, or North American Caucasian). Analyses performed in each of the four study populations were able to identify a unique set of variables which provided excellent discrimination between the two groups (FAS, control).

Objective 5: To better understand the extent to which structural features of FASD are related to specific defects in development of the brain. We have had discussions with Dr. Richard Ward about developing methods to quantify the size of the labial frenulum, a structure which we believe is deficient in children with FAS as a function of the effect of alcohol on forebrain development.

VI. Discussion

The largest number of children ever ascertained with prenatal alcohol exposure have been examined by the Dysmorphology Core since the beginning of this Consortium. Through collaboration with the other clinical as well as basic science projects, it should definitely be possible to delineate the total spectrum of defects associated with prenatal alcohol exposure as well as accomplish the other objectives of the Dyamorphology Core.

We continue to have some concerns about the documentation of maternal alcohol exposure in the retrospective cases as well as issues relating to timing and pattern of alcohol consumption. This problem can be overcome with the prospective studies which we believe have incredible potential particularly for documenting issues relating to the total spectrum of defects as well as their correlation with various patterns of alcohol consumption.

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

Though examination of all children entered into the Consortium at all study sites throughout the world, the Dysmorphology Core interrelates with the aims of the overall project as well as directly with all other clinical projects in the Consortium. In addition, Drs Jones, Chambers, Riley, Mattson and Coles have interacted regarding the value of specific alcohol-related structural defects as predictors of specific measures of IQ. Furthermore Drs Jones and Ward have interacted regarding methods that could be used to measure the labial frenulum, a possible predictor of forebrain development.

VII. Plans for Next Year:

As more babies are born in the prospective studies in Moscow and Ukraine, the Dysmorphology Core will spend additional time evaluating those children. In addition we plan to become further involved in the analysis of data that have already been collected in order to accomplish more of the objectives that we have set forth. This will hopefully involve increased collaboration with the neuro-imaging project and the facial-imaging project. In addition some of the specific objectives that we have outline regarding a better understanding of the extent to which structural

features of FASD are related to specific defects in development of the brain will require more interaction with some of the basic science projects.

IX. Publications

X. Posters and Presentations

Progress Report on Developmental Project: Choline Availability and FASD

I. Principal Investigator: Jennifer D. Thomas, Ph.D.

II. Title of Project: Choline Availability and FASD

III. Objectives:

Choline is an essential nutrient, critical for proper brain and behavioral development. Animal studies have shown that choline availability during prenatal and early postnatal development influences CNS development. Specifically, prenatal choline deficiency impairs brain development whereas perinatal choline supplementation can lead to structural, neurochemical and electrophysiological changes that are reflected as long-lasting improvements in cognitive functioning. This developmental project will examine how choline availability influences ethanol's teratogenic effects.

The Specific Aims are as follows:

1. Determine whether choline supplementation during late gestation will mitigate ethanol's teratogenic effects.
2. Determine whether choline deficiency exacerbates ethanol's teratogenic effects.

IV. Methods:

1. Specific Aim 1: The purpose of Specific Aim #1 is to inform Christina Chambers' clinical intervention study, which will identify alcohol-abusing women mid-pregnancy, counsel them to reduce/stop alcohol consumption and then introduce a micronutrient supplementation, including choline supplementation. We have previously reported that perinatal choline supplementation can reduce the severity of ethanol-related behavioral alterations, suggesting that choline supplementation may serve as an effective treatment for fetal alcohol spectrum disorders. To determine whether choline supplementation restricted to late gestations mitigates ethanol's teratogenic effects, pregnant Sprague-Dawley rats are exposed to 6.0 g/kg/day ethanol from gestational days (GD) 5-20, during the equivalent of the first and second trimesters. Pair-fed and *ad lib* lab chow controls are included. Following birth, ethanol-exposed subjects are treated with 15 mg/kg/day choline during only the third trimester equivalent (postnatal days (PD) 1-9) or during the third trimester equivalent and into postnatal development (PD 1-21). In addition, some ethanol-exposed subjects will continue to receive alcohol during the 3rd trimester equivalent whereas others will not. This design will allow us to determine if choline is effective when administered only during the 3rd trimester or if supplementation would need to continue after birth to be effective. This design will also allow us to determine if choline is effective even if a woman continues to drink alcohol during late gestation.
2. Specific Aim 2: To first determine whether choline deficiency exacerbates ethanol's teratogenic effects, we will examine whether prenatal alcohol itself influences maternal and fetal choline levels. Brain, liver and blood tissue will be collected from the pregnant dam and fetuses and tissue will be sent to the laboratory of Dr. Carl Keen for analyses of choline and associated metabolites. Secondly, although choline can be generated through de novo synthesis, dietary levels of choline greatly influence choline levels. Thus, we are examining whether reductions in dietary choline levels exacerbates ethanol's teratogenic effects. Pregnant dams are intubated with 6.0 g/kg/day ethanol from GD 5-20. Pair-fed and *ad lib* lab chow controls are included. One half of subjects are fed a diet with 100% recommended levels of choline, whereas the other half receive a diet with 40% recommended levels of choline, levels that are consistent with those reported in epidemiological studies and are, thus, clinically relevant. Physical, behavioral, and hippocampal development is examined in the offspring. In addition, brain, liver and blood levels of choline and associated metabolites will be measured by Dr. Carl Keen.

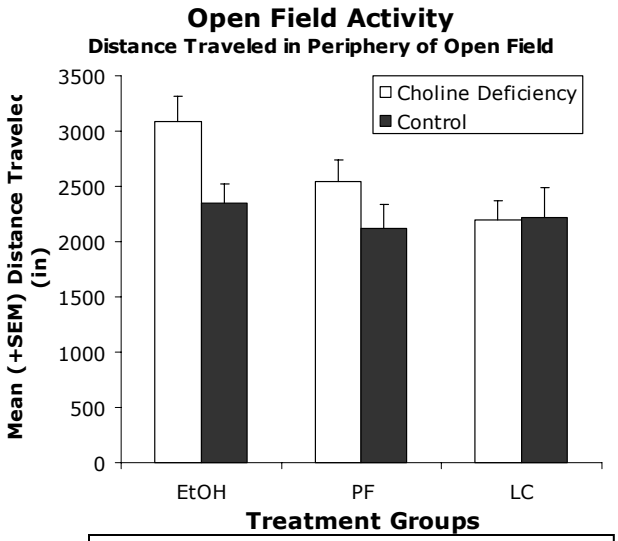
V. Accomplishments and Results:

This project has been funded for 3 months.

1. Specific Aim 1: To date, we have generated 41 experimental subjects, 29 of which have already begun behavioral testing.

2. Specific Aim 2: First, to determine whether ethanol exposure alters choline levels, we have collected brain, liver and blood tissue from four pregnant dams and their fetuses for Dr. Carl Keen's group to run choline analyses before generation of experimental subjects.

Secondly, we have generated approximately 4-5 litters per treatment group in the choline deficiency study. Preliminary data indicate that the effects of choline deficiency depend on the outcome measure. For example, on some behavioral tasks, like open field activity (see figure), behavioral alterations are only observed in alcohol-exposed subjects that also received the choline deficient diet.



EtOH = Ethanol-exposed; PF = Pair fed control; LC = lab chow control

VI. Discussion:

Findings from Specific Aim #1 will help inform both Chambers' clinical study and Cudd's sheep model studies. Identification of the parameters that control the effectiveness of choline on alcohol-exposed subjects is critical for developing choline as a treatment for fetal alcohol spectrum disorders. Examination of ethanol's effects on choline levels and manipulation of dietary choline in the choline deficiency studies of Specific Aim #2 will illustrate how nutritional factors influence alcohol's teratogenic effects. Such data may have relevance to many clinical populations. Elucidation of how nutritional factors, such as choline, can influence the teratogenic effects of alcohol will allow us to better understand risk factors associated with FASD and to develop more effective intervention/treatment strategies.

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

Specific Aim #1 examines the effects of choline supplementation during a period of development in the rat that models late gestation in humans, with the goal of informing the sheep studies of Cudd (U01 Translational Studies of FASD Using a Sheep Model) and the clinical nutritional intervention study proposed by Chambers (U01 Spectrum of and Nutritional Risk Factors for FASD in Russia and Ukraine), both of which include choline supplementation. Specific Aim #2 examines whether choline deficiency exacerbates ethanol's teratogenic effects, complementing Chambers' clinical U01, which will examine blood choline levels in the clinical population. Not only will the animal data inform the clinical studies, but the clinical data will also inform the animal studies to better model clinical conditions.

VIII. Plans for the Next Year:

This developmental project is just beginning and we will continue to collect data on the projects. We plan to complete the experiment for Specific Aim #1 by the end of next year and will also establish whether alcohol itself alters choline levels.

IX. Publications:

None.

X. Posters and Presentations:

None.

Progress Report for Developmental Project: Prenatal Ultrasound and the Early Detection of FASD

I. Principal Investigator: Andrew D. Hull, M.D.

II. Title of Project: Prenatal Ultrasound and the Early Detection of FASD

III. Objectives:

This project builds on preliminary studies performed in Ukraine examining the use of prenatal ultrasound in the assessment of fetuses exposed to alcohol in utero.

Specific Aim #1

To examine the utility of specific ultrasound measurements of fetal brain in the detection of fetuses who will go on to demonstrate features of FASD.

1. To correlate these ultrasound measures with postnatal assessment of growth and structural features as assessed by a standardized dysmorphological examination.
2. To correlate these ultrasound measures with altered neurobehavioral assessment at 6 & 12 months.
3. To assess the dose response relationship between these ultrasound measures and the timing and quantity of alcohol ingested prenatally.
4. To determine the best cut points and gestational age timing for ultrasound measures that produce the most sensitive and specific set of markers predicting 1 - 3 above and the set with the highest positive predictive value.

Specific Aim #2

To assess the role of second and third trimester ultrasound assessment of biophysical profile (BPP) and startle response in the detection of fetuses who will go on to demonstrate features of FASD.

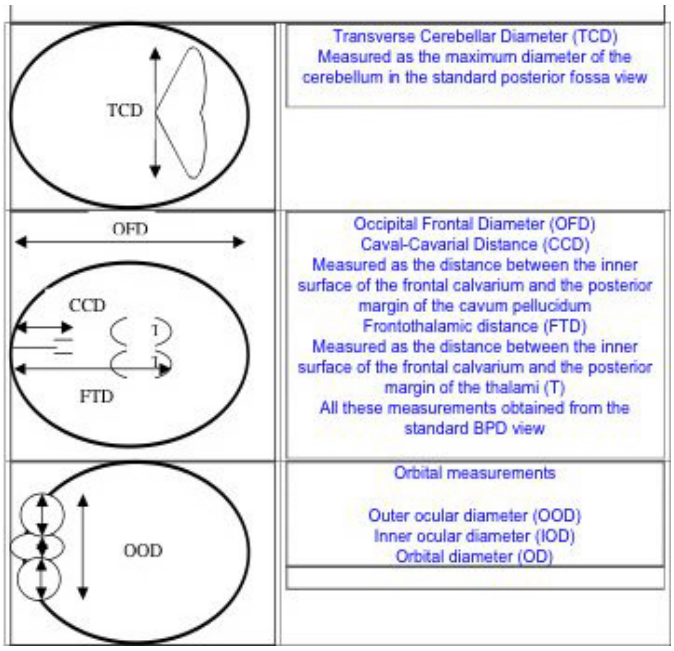
1. To correlate BPP and startle responses with postnatal assessment of growth and structural features as assessed by a standardized dysmorphological examination.
2. To correlate BPP and startle responses with altered neurobehavioral assessment at 6 & 12 months.
3. To assess the dose response relationship between BPP and startle responses and the timing and quantity of alcohol ingested prenatally.
4. To determine the best cut points and gestational age timing for BPP and startle responses that produce the most sensitive and specific set of markers predicting A - C above and the set with the highest positive predictive value.
5. To correlate BPP and startle responses with the ultrasound measures of brain growth in **Specific Aim 1** above.

IV. Methods:

The project continues the preliminary prospective cohort study design at one performance site – the Rivne Oblast in Ukraine. Subject screening, selection recruitment, interviews and record review continue as previously established in the first phase of this project. Serial ultrasound measurements are performed at the Rivne Oblast Regional Diagnostic Center utilizing previously trained ultrasonographers who participated in the CIFASD pilot study in the first three years of the consortium. Subjects are recruited from the cohort of individuals identified as exposed with an equal number of controls as carried out in the pilot study. Women in the exposed and control groups participate in standard ultrasound measures in each trimester conducted by specially trained technicians. Video or DVD recordings of all ultrasound examinations are archived. A total of 300 women are to be recruited (75 per year into each arm for 2 years).

Measurements are obtained just as in the pilot study. Each of the three ultrasound examinations include routine measurements of fetal head circumference, biparietal diameter, abdominal circumference and femur length (BPD, HC, AC, FL). An estimated fetal weight (EFW) is calculated using a standard method according to Hadlock. A detailed fetal anatomic survey is performed at the initial study and targeted anatomic imaging at subsequent follow up studies. All systems are evaluated according to AIUM guidelines for targeted obstetric imaging. Particular attention is paid to evaluation of CNS and cardiac anatomy. All anomalies are noted and recorded. Additional assessments of CNS anatomy and biometry are made as shown in **Figure 1**. Biophysical Profile and Startle responses are new elements of the study. Study sonographers have been taught how to obtain the BPP and use the standard scoring system. Spontaneous and evoked startle responses are also recorded as is fetal heart rate response to an evoked startle. Liveborn infants receive the standard CIFASD dysmorphological examination at either site, and all infants receive a neurobehavioral evaluation using the BSID II at 6 and 12 months of age. An additional neurobehavioral measure involving heart rate monitoring to evaluate attentional regulation has been incorporated. In addition, at 6 months of age, 3-D facial imaging is planned (T Foroud, PI).

Figure 1 Specific CNS Measurements



V. Accomplishments and Results:

1. Since the notice of award, a week long trip was made to Ukraine by the PI to initiate the renewal protocols.
2. The protocol for the additional ultrasound measures of BPP and startle responses has been refined and finalized.
3. The Ukrainian ultrasonographers and sonologists have been trained in the new protocols.
4. Analysis of the prenatal ultrasound pilot data with respect to alcohol exposure have been completed and a publication is in press with ***Ultrasound in Obstetrics and Gynecology.*** See **Tables 1 & 2** below.
5. Analyses of prenatal ultrasound measures and physical features of FASD have also been completed and a manuscript is in preparation.

Table 1. Fetal Brain Measures Assessed During the Second Trimester among Alcohol-Exposed and Control Subjects.

Measures:	**Exposed** (N=66)	**Controls** (N=64)	**p-value**
	Adjusted mean±s.e.	Adjusted mean±s.e.	
Transverse Cerebellar Diameter (mm)	21.8±0.2	22.0±0.2	NS
Occipital Frontal Diameter (mm)	68.5±0.6	68.3±0.6	NS
Caval-Calvarial Distance (mm)	25.6±0.3	26.6±0.4	<0.05
Frontothalamic Distance (mm)	40.5±0.5	41.6±0.5	<0.05
Outer Orbital Diameter (mm)	36.1±0.3	35.9±9	NS
Interorbital Distance (mm)	11.0±0.2	11.3±0.2	NS
Orbital Diameter (mm)	11.5±0.2	11.6±0.2	NS

Table 2. Fetal Brain Measures Assessed During the Third Trimester among Alcohol-Exposed and Control Subjects.

Measure	**Exposed** (N=47)	**Controls** (N=31)	**p-value**
	Adjusted mean±s.e.	Adjusted mean±s.e.	
Transverse Cerebellar Diameter (mm)	41.6±0.4	40.9±0.5	NS
Occipital Frontal Diameter (mm)	106.7±1.4	107.5±1.8	NS
Caval-Calvarial Distance (mm)	42.3±0.6	43.4±0.7	NS
Frontothalamic Distance (mm)	64.2±0.7	67.2±0.8	<0.05
Outer Orbital Diameter (mm)	54.6±0.6	54.7±0.7	NS
Interorbital Distance (mm)	15.6±0.4	14.8±0.4	NS
Orbital Diameter (mm)	16.2±0.3	17.2±0.3	<0.05

VI. Discussion:

The results of the pilot project provide an exciting taste of the full potential of the imaging project. We have demonstrated clear differences in brain morphometry between alcohol exposed and control fetuses in both second and third trimesters. The addition of more subjects will allow objective evaluation of these measures as potential screening tools in an at risk population.

The sonographers will begin the BPP and startle assessments in tandem with morphometric and morphologic measures in the next phase of recruitment. The addition of these novel measures may prove challenging and the PI will visit soon after their introduction to problem solve and confirm correct methodology.

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

The PI has worked closely with Drs Chambers and Jones to integrate the ultrasound protocol with the Dysmorphology Core. The new elements involving BPP and startle responses feed forwards into Dr Coles' neurobehavioral protocols and provide a prenatal component to these assessments.

VIII. Plans for the next year

Recruitment will restart in Ukraine in February. In March the PI plans to visit to reinforce the training carried out during the last visit and develop ideas for further ultrasound studies explored at that time.

IX. Publications:

Jones KL, Hull AD, Bakhireva LN, Wertelecki WW, Yevtushok L, Sosynyuk Z, Shevchenko S, Zharko L, Onishenko S, Drapkina I, Chambers CD. Fetal Ultrasound Measures as Predictors of Alcohol-Related Physical Features in the Newborn: Preliminary Results. (2007) Birth Defects Research Part A: Clin and Molec Teratol .79:373

Kfir M, Hull AD, Yevtushok L, Onishchenko S, Wertelecki W, Bakhireva L, Chambers CD, Jones KL. (2007). Evaluation of Early Markers of Prenatal Alcohol Exposure. Journal of Ultrasound in Medicine. Suppl.1 :740.

Kfir M, Yevtushok L, Onishchenko S, Wertelecki W, Bakhireva L, Chambers CD, Jones KL, Hull, AD. (2007). Can Prenatal Ultrasound Detect the Effects of In utero alcohol exposure? Ultrasound in Obstetrics and Gynecology 30: 423, OC182.

Kfir M, Yevtushok L, Onishchenko S, Wertelecki W, Bakhireva L, Chambers CD, Jones KL, Hull AD Can prenatal ultrasound detect the effects of in utero alcohol exposure? – A pilot study. Ultrasound in Obstetrics and Gynecology (In press).

X. Posters and Presentations:

M. Kfir, A. Hull, L. Yevtushok. S. Onishchenko, W. Wertelecki, L. Bakhireva, C. Chambers, K. Jones. Evaluation of Early Markers of Prenatal Alcohol Exposure. Oral presentation at the American Institute of Ultrasound in Medicine Annual Convention. March 15-18, 2007. New York, NY.

Kfir M, Yevtushok L, Onishchenko S, Wertelecki W, Bakhireva L, Chambers CD, Jones KL, Hull, AD. (2007). Can Prenatal Ultrasound Detect the Effects of In utero alcohol exposure? Oral presentation at 17th World Congress on Ultrasound in Obstetrics & Gynecology held in Florence, Italy, October 7 – 11 2007. *

* Awarded prize for best oral presentation at meeting.

Progress Report for Translational Studies of FASD Using a Sheep Model

I. Principle Investigator: Timothy A. Cudd, D.V.M., Ph.D.

II. Title: Translational Studies of FASD Using a Sheep Model UO1 AA017120

III. Objectives:

The Specific Aims are as follows:

1. Test the hypothesis that binge-like prenatal alcohol exposure in the 1st-trimester model and the 3-trimester model will produce facial dysmorphology and reduced brain volumes (microencephaly), but that the effects on brain growth are predicted to be more severe in the 3-trimester model. Morphometric analysis of facial structure will be performed at three postnatal ages, and 3-dimensional structural MRI will be performed at 3 months of age for quantitative analysis of overall brain volume and of volumes of specific brain regions.
2. Test the hypothesis that deficits in cerebellar-dependent and hippocampal-dependent learning and memory will be more severe in the 3-trimester model than in the 1st-trimester model, and that these effects will be associated with more severe cerebellar and hippocampal neuronal loss (from Aim #3). Pavlovian "delay" eyeblink condition will be used to assess cerebellar-dependent learning; Pavlovian "trace" eyeblink conditioning will be used to assess learning that requires both cerebellar and hippocampal function; spatial delayed alternation will also be used to assess hippocampal-dependent spatial working memory.
3. Test the hypothesis that both the 1st-trimester and the 3-trimester models will produce deficits in neuron numbers in the cerebellum and hippocampus, but that loss of hippocampal and cerebellar neurons will be more severe in the 3-trimester model. Stereological counts will be obtained for cerebellar Purkinje neurons, deep cerebellar nuclei neurons, hippocampal pyramidal neurons of CA1 and CA3, and granule cells of the dentate gyrus, for analysis of group differences and for correlation with behavioral performance from Aim 2. Based on previous findings in mice that gestational alcohol exposure produces deficits in serotonin neurons, counts of serotonergic neurons in the raphe will be included.
4. Test the hypothesis that choline supplements in the 3-trimester model, beginning shortly after mating, will significantly reduce the effects of alcohol exposure on behavioral and brain outcomes. Facial morphology, structural MRI, eyeblink conditioning and spatial learning, and neurohistological analyses similar to Aims 1-3 will be used to assess whether choline supplements protects against this full 3-trimester exposure.

IV. Methods:

As described above, we will utilize the sheep to model all three trimester alcohol exposure. We will utilize structural measures (MRI and cell counting in the cerebellum and raphe) and functional measures (eyeblink classical conditioning to assess cerebellar and hippocampal injury and T maze to assess hippocampal injury). We will utilize physical and possibly photographic and MRI imaging to assess whether the sheep model exhibits facial dysmorphology. We will utilize physical and MRI measurements to assess brain and brain component volumes and architecture. And finally, all of these measures will be utilized to determine if choline supplementation in the sheep all three trimester model is protective. All of these measures closely parallel those being used or that are being investigated in clinical subjects but with the advantage in the sheep model that the independent measures can be controlled and dependent measures can be correlated to post mortem findings.

V. Accomplishments and Results:

This project began in September, 2007 and we have now bred and enrolled the first contingent of animals (note that gestation in this species is 147 days). These animals are one half of the subjects necessary for the completion of Specific Aims 1, 2 and 3. The collaborators on this project have begun the process of establishing the new

dependent measures for this project, the facial and MRI measurements. Lambs will be born in the spring after which the dependent measure collection will begin.

VI. Interrelation with Aims of the CIFASD and Other Projects:

This project will contribute to the advancement of the six general CIFASD themes/aims in the following ways:

1) To further the goal of establishing standardized diagnostic criteria and methods of assessing FASD, the sheep will parallel clinical efforts, utilizing methods that are as similar as possible to those used in human subjects. However, in the sheep, the timing and dose of exposure will be precisely known, an advantage that will potentially strengthen and clarify the findings in human subjects.

2) To enhance the understanding of the neurobehavioral phenotype of FAS and AR ND the sheep model will utilize eyeblink classical conditioning and T maze learning in subjects where the exposure dose and pattern is precisely known and where all other environmental conditions can be controlled for, an advantage that could potentially strengthen and clarify findings in human subjects.

3) To enhance understanding of FASD dysmorphology through 2D and 3D image analyses, we will work closely with the clinical components of the consortium to determine if dysmorphology is expressed in the sheep. At present, there is no ideal animal model of FASD dysmorphology. The development of models of FASD dysmorphology will potentially assist in the development of more sensitive and accurate means of identifying affected individuals and will aid in the development of preventative strategies.

4) The characterization and correlation of structural and functional neurobehavioral deficits will be performed in this model system, using MRI, cell counting, and functional measures, where the dose and timing of exposure is precisely known. These studies will potentially strengthen clinical studies where this information is not available.

5) Efforts to improve early case identification will potentially be enhanced by this project by utilizing the dependent measures that can be utilized in human subjects to screen for FASD in a model system where the independent measures can be precisely controlled and where complete post mortem findings can support and confirm imaging and functional findings.

6) This project will test an intervention (choline supplementation) designed to reverse or ameliorate neurobiological deficits. This approach is currently being assessed in a rodent model in another component of the consortium. However, the advantage of the sheep model over the rodent model is that all three trimester equivalent of human brain development occur in utero, as in humans.

VII. Plans for Next Year:

We plan to complete experiments on one half of the subjects necessary to complete specific aims 1-3.

VIII. Publications:

None

IX. Presentations:

October, 2007: Invited speaker, Bowles Center for Alcohol Studies, University of North Carolina School of Medicine, Chapel Hill, NC. “In Utero Ethanol Exposure Results in ARND: Use of the ovine model to explore mechanisms of action, detection and amelioration strategies.”

Progress Report for Photolabeling of Alcohol Binding Sites on L1

I. Principal Investigator: Keith W. Miller, Ph.D.; Subcontract to Michael Charness, M.D.

II. Title: Photolabeling of alcohol binding sites on L1

III. Objectives:

The Specific Aims are as follows:

1. 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.
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) or 3-azibutanol (100-1000 μM), incubated for 10 minutes in the dark and then irradiated for 30–40 minutes at 365 nm. 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 using FT/MS/MS spectrometer for sequencing of fragments. Mass spectra are acquired from m/z 300 to 2000 with a maximum ejection time of 400 ms. 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:

1. Specific Aim 1: 3-Azibutanol binding sites were identified on the purified Ig1-4 fragment of human L1 (hL1 Ig1-4). At 50 μM 3-azibutanol, no photolabeled tryptic peptides were detected, whereas at 100 μM and 1 mM 3-azibutanol, one and four such peptides, respectively, could be reproducibly sequenced. Trypsin digestion enabled the sequencing of 87% of the residues within hL1 Ig1–4, and chymotrypsin digestion extended this coverage to a total of 95%. The single peptide identified after labeling with 100 μM 3-azibutanol was analyzed after trypsin digestion and demonstrated photolabeling at Tyr-418. A second labeled peptide identified after chymotrypsin digestion showed photolabeling at Glu-33 and along the sequence from Glu-24 to Glu-27.
2. Specific Aim 2: At a high concentration of 1 mM 3-azioctanol, twelve photolabeled tryptic peptides were identified by mass and six of them were reliably sequenced. In contrast, at 10 μM 3-azioctanol, only one photolabeled tryptic peptide was detected. Strikingly, this peptide demonstrated labeling at Tyr-418, the same residue identified by the agonist 3-azibutanol. A second peptide identified after chymotrypsin digestion showed labeling at Glu-33 and Glu-24 to Tyr-26, but did not strongly discriminate among these two sites. Photolabeling at Glu-33 was confirmed using mouse, rather than human, L1 Ig1-4.
3. Homology Model: Because there is no crystal structure for L1, we constructed a homology map using axonin-1. The model's most notable feature is an almost globular, or horseshoe-shaped structure, in which Ig1 lies opposed to Ig4 and Ig2 lies opposed to Ig3 in antiparallel fashion. This folded structure is enabled by a linear stretch of amino acids between Ig2 and Ig3, shown in purple, that is unique to the L1 family of cell adhesion molecule. The model predicts that two photolabeled residues, Glu-33 and Tyr-418, though widely separated on the primary sequence, are situated in close proximity at the domain interface between Ig1 and Ig4. Indeed, the e1 oxygen of Glu-33 lies just 2.8Å from the h oxygen (hydroxyl) of Tyr-418, forming a strong hydrogen bond. This alcohol binding pocket also abuts two residues, Leu-120 and Gly-121, at which missense mutations cause disease.
4. Protein production**:** We have begun efforts to crystallize hL1 Ig1-4 using a nanoliter robotic system. We initially purified to high concentrations a construct from HEK293 cells containing hL1 Ig1-4-TEV-Fc, but

initial efforts at crystallization were unsuccessful. We have subsequently switched to a bacterial expression system to avoid post-translational glycosylation, which can sometimes interfere with protein crystallization. We have successfully purified from *E Coli* large quantities of hL1 Ig1-4-TEV-Fc and will begin shortly new efforts at crystallization.

VI. Discussion:

Electron microscopic studies reveal both a linear and a horseshoe conformation for the first six Ig domains of human L1. Haspel and Grumet have proposed that the Ig1–Ig4 domains of L1 interconvert between the horseshoe and linear conformation, with homophilic binding favored by the horseshoe conformation. The potential importance of the horseshoe structure of L1 is underscored by the conservation of residues across species at the domain interfaces between Ig1 to Ig4 and Ig2 to Ig3 and the disproportionate number of these residues in which missense mutations cause developmental abnormalities of the nervous system.

Binding of ethanol to the Glu-33–Tyr-418 pocket could perturb L1 function in several ways. First, ethanol could inhibit homophilic binding by disrupting one of the four hydrogen bonds between Ig1 and Ig4 that stabilize the horseshoe conformation of L1. Second, the Glu-33–Tyr-418 binding pocket lies in close proximity to two disease–causing residues: Leu-120 and Gly-121. The side chain d methyl group of Leu-120 is only 4.7 Å from the a carbon of Glu-33 and 6.7 Å from the aromatic ring of Tyr-418, distances that are comparable to the lengths of extended ethanol (3.75 Å) and octanol (10 Å) molecules. The Leu-120-Val mutation results in the loss of Sema3A regulation of L1-neuropilin interactions, but does not affect L1 homophilic binding. On the other hand, the Gly-121-Ser mutation reduces L1 homophilic binding by 90% and decreases L1 interactions with FII and contactin. Thus, the binding of ethanol in the region of these disease-causing mutations could disrupt development by inhibiting homophilic or heterophilic interactions of L1.

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

This project is one of the Consortium's basic science projects. We have accomplished our principal of demonstrating that there are alcohol binding sites on L1. These findings establish the intellectual framework for seeking pharmacological antagonists to ethanol based on the structure of an alcohol binding site. In this regard, the project advances the Consortium's goal of identifying new medications to prevent FASD.

VIII. Plans for Next Year:

Our competitive renewal was not funded. During the next few months, we will begin some of the experiments outlined below using funds from other grants. Thereafter, we will no longer be able to support our structural chemist.

1. New Specific Aim 1: Crystallography. Characterize the three-dimensional structure of ethanol agonist and antagonist binding sites by crystallization of L1. We will begin by crystallizing the first four Ig domains of L1, where photolabeling data have localized potentially important agonist and antagonist binding sites. We will screen using nanoliter screening high throughput crystallization technology at the Hauptman-Woodward Medical Research Institute (http://www.chtsb.org/center/hwi.php). The nanoliter screening crystallization trials will be carried out with commercially available sparse matrices (Hampton Research, CA and/or Emerald Biosystems, WA). The main variables will be pH (4–9), the type of precipitant (e.g. polyethylene glycol (PEG) or salts), and the precipitants' concentration. Once crystallization conditions are established, we will use the hanging drop vapor diffusion method, routinely employed in our laboratory, to produce larger crystals for diffraction experiments. Normally alcohols in the crystallization solution diffuse into preformed crystals (our standard method). Alternatively crystals can be grown in the presence of alcohols. This has also been successful in our hands.
2. New Specific Aim 2: Determine the role of the alcohol binding sites on L1 structure and function. The interface between Ig1 and Ig4 of hL1 is a critical location for hL1 function. We have identified an alcohol binding pocket within this domain interface. We will employ mutational analysis of residues within the alcohol binding pocket to determine the role of individual residues in L1 function, alcohol inhibition of L1 adhesion, and antagonism of alcohol inhibition of L1 adhesion. L1 constructs will be expressed in HEK293 and NIH/3T3 cells and purified for analysis. The effects of expressed proteins on L1 function will be determined. If mutations do not impair L1 function, then the effect of mutations on alcohol inhibition of L1 adhesion and antagonism of alcohol effects will be determined. We also determine the effect of single mutations on photolabeling of hL1 Ig1-4 by 3-azibutanol and 3-azioctanol.

IX. **Publications:**

Arevalo, E., Shanmugasundararaj, S., Wilkemeyer, F., Dou, X., Chen, S., Charness, M. E., Miller, K. W. (2008). An alcohol binding site on the neural cell adhesion molecule L1. *Proc Natl Acad Sci (USA),* 105, 371-375.

X. Posters and Presentations:

2007 "Alcohol and the L1 Cell Adhesion Molecule" Winter Conference on Brain Research, Snowmass, CO

2007 "Overview: Fetal Alcohol Spectrum Disorders" NIDA Workshop: Intrauterine Exposure to Drugs and Alcohol: What is the next step for Medication Research & Treatment?" Bethesda, MD

2007"Alcohol and the L1 Cell Adhesion Molecule", Department of Pharmacology Seminar, Medical College of Virginia, Richmond, VA

2007"Alcohol and the L1 Cell Adhesion Molecule", Ernest Gallo Clinic and Research Center, UCSF, Emeryville, CA

2007"Alcohol and the L1 Cell Adhesion Molecule", Department of Neuroscience and Physiology, State University of New York, Syracuse, Syracuse, NY

2007"Alcohol and the L1 Cell Adhesion Molecule", Center for Drug and Alcohol Programs, Medical University of South Carolina, Charleston, SC

Progress Report for Mouse Magnetic Resonance and Diffusion Tensor Imaging

I. Principal Investigator: Kathleen K. Sulik, Ph.D.

II. Title of Project: Magnetic Resonance and Diffusion Tensor Imaging of a Mouse FASD Model

III. Objectives:

The primary objective of this work is to inform the establishment of improved diagnostic criteria for FASD and to make new discoveries regarding the impact of prenatal ethanol exposure on the brain and face. Utilizing a mouse FASD model, critical periods of ethanol-induced teratogenesis, with emphasis on embryonic and early fetal periods of development, are being assessed. Magnetic Resonance Imaging (MRI) and Diffusion Tensor Imaging (DTI) are being employed to allow detailed analyses of brain and facial structures and correlation of abnormalities of the brain and face within single individuals, as well as comparison among experimental groups. The results of this work will be compared to corresponding analyses of human subjects in addressing our overall hypothesis that ethanol induces structural abnormalities of the brain and face of mice that are consistent with and informative for those in human FASD.

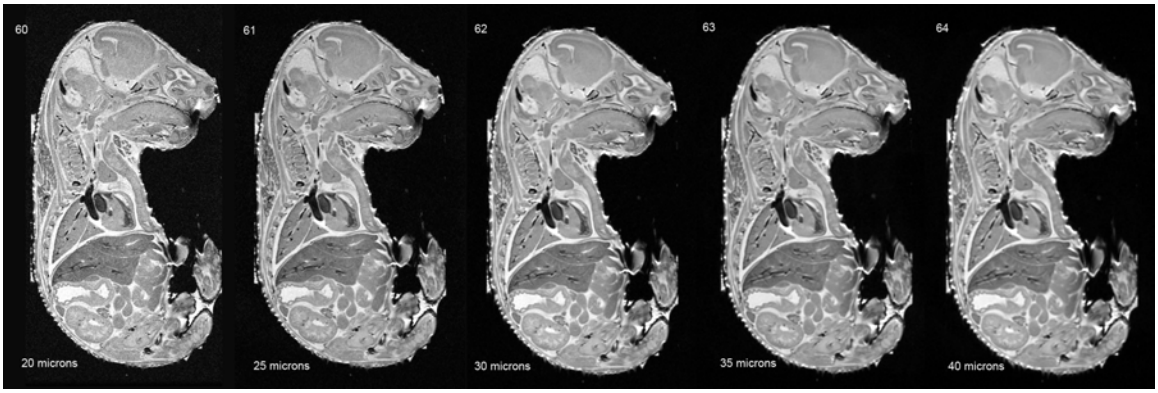
IV. Methods:

High resolution MRI and/or DTI methodologies are employed for all of our proposed work. Both individual scans and 3-D reconstructions derived from them will be analyzed to provide data regarding developmental stage and dose-dependent dysmorphology of the brain and face of ethanol-exposed fetal mice. In order to efficiently utilize our resources, a subset of fetuses will be collected in our laboratory, while another subset will be collected in Dr. Zhou's laboratory. Pregnant mice will be acutely administered alcohol via either intraperitoneal injections or via liquid diet on GD 7, 8, 9, 10, or 11, or will be chronically administered alcohol over the period of GD7-11, 12-16, or 7-16. All fetuses will be collected on GD 17, fixed in a Bouins + Prohance solution and delivered to Duke University's Center for In Vivo Microscopy (CIVM) for imaging. Imaging data will be sent to our laboratory electronically for segmentation, reconstruction, and data analyses. Linear, volume and/or area measurements for a number of selected brain and facial regions will be made, with duplication of data analysis for each measure.

V. Accomplishments and Results:

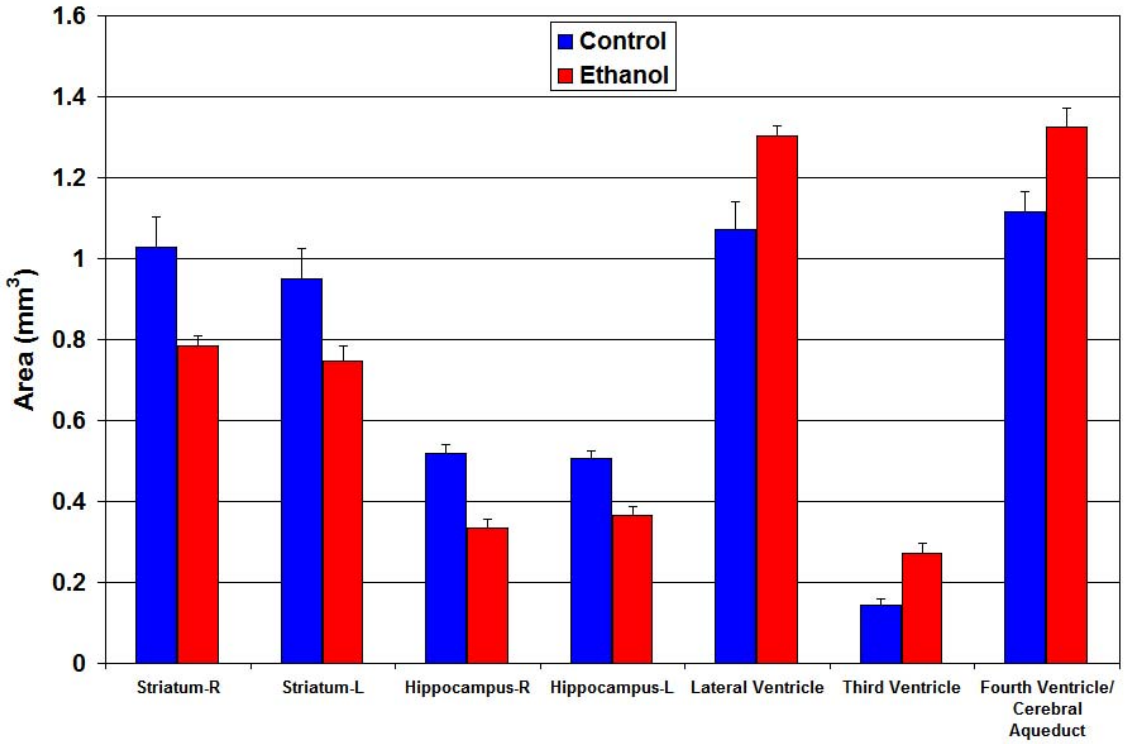
In the 3 month period since this project was funded, we have:

1. Determined optimal conditions for imaging our specimens at CIVM. Illustrated below is a panel comparing image resolution obtained using the CIVM 9.4 T magnet. We determined that 25 microns provides the best combination of resolution and tissue contrast. This is the resolution at which data will be acquired for this study.



2. Duplicated segmentations and began analysis of CNS data from fetuses that had been acutely exposed to ethanol on GD 7 or 8, and initiated collection of the GD 9 acute exposure group. GD 7 exposure resulted in a spectrum of CNS abnormalities, with a major site of deficiency being the rostral and ventro-medial forebrain; i.e. the septal region, olfactory bulbs and caudate/putamen of GD17 fetuses. Illustrated in the

figure below, are data from the brains of acute GD 8-exposed fetuses. Notable differences between the ethanol-treated and control fetuses were observed in the hippocampus, caudate-putamen (striatum) and the third ventricle. The hippocampi and caudate-putamen of ethanol-exposed fetuses were smaller than controls, while the third ventricle was significantly enlarged after ethanol exposure as compared to controls.



3. Initiated studies with the Zhou group that are designed to assess possible differences between ethanol's teratogenesis in the 2 different lines of C57Bl mice that have traditionally been used in each of our laboratories. This work is being conducted in order to determine whether we need to adopt a single mouse line for the planned joint experiments.

4. Hired a postdoctoral fellow, Dr. Shonagh O'Leary Moore, who will have primary responsibility for our proposed DTI analyses. She will begin by working closely with Dr. Yi Jiang at CIVM to maximize parameters for collection of data from our fetal mice. As illustrated in a recent DTI image prepared by Dr. Jiang, the CIVM group has successfully applied this technology to illustrate fiber tracts (shown in green) in the rodent brain.

VI. Discussion:

It is clear that the MRI and DTI work that we have initiated holds great promise for facilitating identification of the spectrum and temporal-dependence of alcohol-induced brain dysmorphology. Of particular interest from our most recent analyses is that maternal ethanol exposure on GD8 in mice (corresponding to the fourth week of human prenatal development) results in hippocampal deficiencies. Prior to this, research regarding the hippocampus as a site of alcohol teratogenesis has focused on exposure at much later (perinatal) developmental stages. Implications for alcohol-induced behavioral and cognitive deficits occurring prior to the time that most pregnancies are recognized are tremendous. Although our imaging studies of rodent brains have only just been initiated, they are providing important new data from which to draw for the study of human FASD subjects.

VII. Interrelation with the Consortium and Other Projects:

Our high resolution MRI analyses of the CNS compliment those human MRI studies being conducted by Dr. Sowell and the ultrasound project of Dr. Hull, while the facial reconstruction work compliments that employing 3D images as are being prepared and analyzed by Dr. Foroud's group. Additionally, our mouse model work correlates with that of Dr. Cudd, which employs MRI analyses of a sheep FASD model. In testing our main hypothesis, we are working directly with Dr. Zhou's laboratory to produce and assess ethanol-exposed C57Bl mice.

VIII. Plans for the Next Year:

During the remainder of this funding period, we hope to:

1. Complete our analyses and report our CNS MRI data for the acute GD 7 exposure group.
2. Conduct DTI data analysis for the acute GD7 exposure group.
3. Begin analyses of facial morphology in the acute GD7 and 8 exposure groups.
4. Determine whether the 2 C57Bl mouse lines have a comparable incidence of eye defects as an indicator of their similarity of teratogenic response to ethanol.
5. Collect fetuses for the GD 7-11 200mg/dl chronic exposure group.

IX. Publications:

We are in the process of preparing our first MRI-based manuscript. It will be first authored by Dr. Scott Parnell and will describe the result of acute GD8 ethanol insult on the fetal mouse brain.

X. Posters and Presentations:

Kathy Sulik, Ph.D. presented high resolution MRI results regarding the CNS effects of acute gestational day 7 versus day 8 ethanol exposure at a NIAAA/INSERM meeting regarding US / French Research Collaborations in Alcohol Abuse and Alcoholism. The meeting was held at NIAAA headquarters in Rockville MD on Oct 2, 2007.

Liz Myers, our developmental toxicology graduate student, presented the results of her MRI work to date for a UNC Toxicology Department Seminar on Nov 19, 2007.

Kathy Sulik presented lectures to UNC Pathology graduate students (Nov 16) and first year medical students (Dec12) that included description of the results of our studies to date.

Progress Report for Therapeutic Agents

I. Principal Investigators: Feng C. Zhou, Ph.D. (AA014829S and U01 AA017123); Charles R. Goodlett, Ph.D. (U01 AA014829)

II. Title of Project: Testing FASD Therapeutic Agents: Neonatal Rodent Models U01 AA014829 (Goodlett) and AA014829S (Zhou)
New Project: Mouse Model Neuro-Facial Dysmorphology: Translational and Treatment Studies U01 AA017123-01 (Zhou)

III. Objectives:

1. U01 AA014829, Goodlett**:** 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).

2. AA014829S, Zhou**:** The long-term goal of this project is to test therapeutic agents that may protect against fetal alcohol spectrum disorders (FASD). The objectives of the phase I studies are to examine how the activity dependent neurotrophic protein (ADNP) L-form peptide analogues NAPVSIPQ (NAP) or SALLRSIPA (SAL) of Activity Dependent Neurotrophic Protein protect against : (Aim 1a) the deficit in the deficit in neural tube and forebrain development, and (Aim 1b) the deficit in midline brainstem and the 5-HT neurons formation, induced in prenatal models of alcohol exposure; and (Aim 2) apoptosis in the neonatal model of alcohol binge exposure.

IV. Methods:

1. U01 AA014829, Goodlett**:** Our previous progress reports have detailed the lack of effect of vitamin E or L-NAP in protecting against structural or functional damage the cerebellum induced by binge-like neonatal alcohol exposure in rats, using “delay” eyeblink conditioning as the primary behavioral task.

   In the past year, we have completed studies in B6 mice treated with a heavy binge dose of alcohol (2.5 g/kg per injection, two injections, 2 hours apart), given either on postnatal day (PD) 7 or on PD 7-9 (matching the treatments used in Dr. Zhou’s studies of caspase-3 activation associated with apoptosis, described below). Groups of mice were given alcohol either on PD7 or PD7-9; controls were either given saline (PD7-9) or were reared normally (in separate litters). Mice were tested either as juveniles (PD30-40) or as adults (PD 65-75) on place learning in the Morris water maze (4 trials per day for 10 days, with a probe trial at the end of the 5^{th} and 10^{th} day of training).

2. AA014829S and U01 AA017123, Zhou:

   a. Aim 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 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 SAL (ALC+SAL, 20µg/day, i.p.), or NAP (ALC+NAP, 20µg/day, i.p.). The embryos were taken at E15 and the midline structure was

analyzed and the morphology and number of the serotonin neurons were analyzed.

b. Aim 2. Testing the protective effect of NAP against alcohol exposure induced apoptosis in the limbic brain and striatum: Concurrent single-day or three-day alcohol exposure (two s.c. injections, 2 hrs apart, of alcohol, 2.5 g/kg/injection/day) with NAP treatment (20 μg/day, i.p.) or vehicle treatment (vehicle treatment control) were performed in neonatal B6 mice (beginning on postnatal day 7). Animals were perfused at the end of concurrent alcohol exposure and NAP/Vehicle treatment for immunocytochemical staining of caspase-3, an indicator of apoptosis. A scale of the number of caspase+ neurons was used as quantitative index of alcohol-induced apoptosis and protective of NAP.

c. New Preliminary Study 1. Effects of NAP or SAL effect on facial dysmorphology in mouse model: The liquid diet model of prenatal alcohol exposure in B6 mice was used as described for Aim 1 above. The embryos at E15 were taken and photographed and a 2-D morphometric analysis was performed for facial features such as head size, eye distance, and nose size were explored.

d. New Preliminary study 2. New approach for diagnosis of facial dysmorphology using Micro-CT: Using scanner EVS-R9 system (Enhanced Vision Systems Corp, London, Ontario), Micro-CT images were acquired for embryos postnatal day 1 (P1). The image acquisition was performed in-vivo with animals being anesthetized with either Isoflurane or Avertin. When using Isoflurane, the animal will be placed in a induction chamber with Isoflurane levels of 1-1.5 % at a initially flow rate of 0.8-1.2 liter/min followed by a maintenance rate of 0.4-0.8 liter/min for approximately 30-45 minutes required for the Micro-CT scan.

V. Accomplishments and Results:

1. U01 AA014829, Goodlett**:** As juveniles, the PD 7-9 group, but not the PD7 group, showed significant deficits in place learning and memory. Interestingly, as adults, both the PD 7 and the PD 7-9 groups showed significant deficits relative to the two control groups. These results suggest that the structural damage to hippocampal formation and limbic cortex (see Results from Zhou, below) induce long-lasting effects on spatial learning and memory, and this model may be usefully applied to other potential therapeutic agents.

   We have also demonstrated that neonatal binge alcohol exposure in rats induces deficits on "trace" eyeblink conditioning, 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. Ongoing studies in mice are assessing whether the PD 7 and PD 7-9 treatments induce significant effects on trace eyeblink conditioning, as predicted by the damage to hippocampal-forebrain circuits.

2. AA014829S and U01 AA017123, Zhou:

   a. Both SAL and NAP protects against alcohol-induced on neural tube deficit and growth retardation and microencephaly and effects on body weight, brain weight, and brain volume.

   Prenatal alcohol exposure was produced via consumption of a liquid diet containing 25% ethanol-derived calories (EDC) on GD7-15 (or GD7-18) by C57BL/6 dams, producing blood alcohol concentrations (BACs) ~70-140mg/dL. Pair-fed (PF) controls were given isovolemic, isocaloric liquid diet matched to the alcohol group with maltose-dextrin substituted for alcohol. Prenatal alcohol exposure (ALC) designates this liquid diet model unless otherwise mentioned. This mouse model reliably induces the typical reduction of body weight, head size, brain weight and volume. Major gross brain deficits include underdevelopment of brain areas, cortical thinning, ventricle enlargement, and restricted midline neural tissue growth leading to openings at the roof/floor plate. The SAL treatment prevented the reductions of fetal body weight, brain weight, brain volume, and regional brain size. Furthermore, SAL prevented the alcohol induced deleterious effect including cortical thinning, neural tube openings (both size and frequency), and ventricular enlargement. The ability of SAL to antagonize alcohol-retarded brain growth and development of the forebrain and midline neural tube at midgestation suggests its potential use as an antagonist against fetal alcohol-induced microencephaly early in development (Zhou et al., 2004).

Similar to SAL, the NAP treatment increased the fetal body weight ($p<0.01$) of alcohol-treated (ALC) group compared to ALC groups without NAP, and prevented fetal brain weight reductions ($p<0.01$) of the ALC group, rescuing brain weights to levels comparable to PF and chow-fed (Chow) control groups. NAP pretreatment also prevented the forebrain volume reduction in the ALC group ($p<0.01$) to the level comparable to those of PF and Chow control groups. NAP protected against prenatal alcohol-induced reductions in the size of several brain regions, including the ganglionic eminence, diencephalon, septal nucleus, hippocampus, and amygdala, again achieving levels similar to those of PF and Chow group. Fetal alcohol exposure also reduced the thickness of the medial frontal and cingulate cortices, in agreement with previous observations of 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 increased thickness in the differentiating zone of cortex, but not the germinal zone. No difference was found between PF and Chow in the above parameters (Manuscript in submission).

Neural Tube Opening and Ventricle Enlargement. Fetal alcohol exposure reduced cell mass in the neural tube and the timely formation of midline tissue particularly in the roof and floor plate. The thinning of floor and roof plates led to intermittent openings in the neural tube especially in the regions of future lateral and 3rd ventricles (Zhou et al., 2003a). Both SAL (Zhou et al., 2004) and NAP increase the cell mass and thus reduce the size and frequency of the roof and floor opening. Fetal alcohol exposure enlarged the 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 3rd ventricle.

Protection against alcohol-induced reductions in serotonin neurons: The GD15 fetal 5-HT-im neurons were counted through the rhombencephalon which contains 5-HT neurons in the entire rostral raphe. In this range of tissue, the ALC had an average of 20-25% 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, 2002; Zhou et al., 2003a). No statistical difference was found between PF and Chow groups, or between Chow and NAP/SAL+Chow groups. Between the ALC+NAP group and the ALC+SAL group, the SAL significantly increased the number of 5-HT-im neurons from the level of the ALC group to the level of PF and Chow groups (manuscript submitted).

In the caudal raphe containing descending 5-HT neurons, there was an average of 20-25% fewer 5-HT-im neurons in ALC groups as compared to those of Control and PF groups. The NAP treatment (NAP+ALC) increased the number of 5-HT neurons toward the normal level as the Chow and PF groups.

b. Lack of protection against alcohol-induced apoptotic injury of the hippocampal and cortical neurons: NAP treatment is stage sensitive. We have demonstrated that a single day (P7) of alcohol exposure with two injections of 2.5 g/kg (s.c.), 2 hours apart, produced significant apoptosis in the medial cingulate cortex, limbic system, and striatum, identified immunocytochemically by the apoptotic marker active-caspase-3. The treatment with NAP (2 μg/day, s.c., concurrent with the alcohol administration) failed to reduce the active-caspase-3 marker. Nor did NAP treatment (P7-9) concurrent with a 3-day alcohol exposure, protect against the alcohol induced apoptosis as indicated by caspase+ neurons in the above brain regions.

c. NAP or SAL has potential in protecting alcohol-induced mouse facial and body developmental deficit: Prenatal alcohol exposure in the mouse model reduced head size (width), eye distance, and nose width in the ALC group as compared to the Chow and PF groups. Both SAL and NAP (s.c., 2 μg/dam/day throughout the days of alcohol treatment) protected against the reduced head size and nose width, but did not protect against shorter eye distance. No difference was found between PF and Chow groups in the above parameters.

There were significantly increased frequencies of gross malformation or missing eyes in the ALC group as compared with PF and Chow (**Table 1**). Both SAL and NAP (s.c., 2 μg/dam/day throughout the days of alcohol treatment) protected against the effect of alcohol-increased gross eye malformation. The ALC group had significantly increased deformation of limbs, and both ALC and PF groups had delayed segregation of digits (webbed digits) as compared with Chow groups. Both NAP (ALC+NAP) and SAL (ALC+SAL) protected against this alcohol or dietary effect. The number of abnormalities seen (missing limbs, missing digits, undifferentiated digits and malformations of the eye) were higher in the alcohol group and usually occurred unilaterally, though in the case of webbing of the digits the results were

generally bilateral. These effects were not prevented by a scrambled peptide (random sequence) of SAL but were prevented by the combination of ADNF peptides NAP and SAL.

Table 1. The protective effect of NAP and SAL on alcohol-induced peripheral organ dysmorphology.

	Missing Limb		Missing (Budd)		Webbed Digit		Eye malformation		n
	Single	Bilateral	Single	Bilateral	Single	Bilateral	Single	Bilateral	
Chow	0	0	8*	0	6	0	3	0	34
Chow + SAL	0	0	0	0	0	0	0	0	17
Chow +NAP	0	0	0	0	0	0	4	0	25
PF	0	0	0	0	0	10**	0	0	30
Alc	10.3	0	3.4	13.8	3.4	20.7	10	0	29
ALC+Scramble	6.9	3.4	0	0	17.3	24.1	6.9	0	29
ALC+SAL	2.7	0	2.7	0	5.4	0	2.7	0	37
ALC+NAP	4.3	0	4.3	0	0	4.3	0	0	23

*,** : Group derived from same litter

4. Micro-CT images of cranial-facial structure at P1.

The cranial structure shown in figure 1 is segmented with a threshold of 800 HU (Hundsfeld Unit). This threshold is typical for mineralized bone tissue (right panel). Micro-CT imaging also has limited soft-tissue contrast resolution. Although it is difficult to differentiate the soft tissues, the outer surface of the facial skin can be mathematically outlined by a simple thresholding. The facial thickness, computed as the distance between the facial surface and cranium may be used as an additional evaluation of craniofacial feature in FAS (Figure 1, left panel).

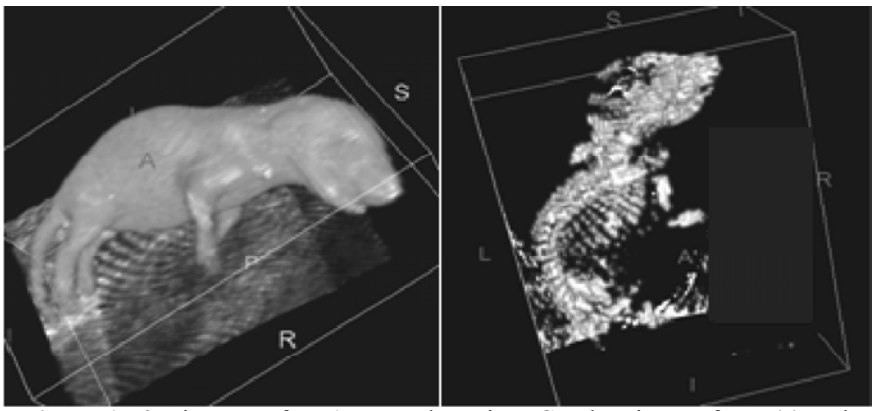


Figure 1. 3D images of a P1 mouse by Micro-CT showing surfaces (a) and skeletons (b) respectively.

VI. Discussion:

The protective effects of NAP/SAL on brain development and, preliminarily, on craniofacial development, support the contention that these agents may have therapeutic potential for pregnancies at risk for FASD. The studies in the new funding period are designed to address key questions related to dose and timing of the alcohol exposure on craniofacial and brain development in prenatal mouse models. These studies will provide essential information for the subsequent studies of the protective effects of NAP/SAL, addressing the long-term outcomes in the prenatal mouse model.

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. It is still of interest to determine whether other treatments may have some beneficial effects on hippocampal/cortical function, given our demonstration that 1-day and the 3-day alcohol binge exposure in B6 mice results in permanent deficits spatial learning.

VII. Interrelation with Aims of the Consortium and other projects:

1. The effect of prenatal NAP and SAL on alcohol-induced deficits will continue to be assessed in consultation and collaboration with Dr. Michael Charness. The change to a focus on prenatal exposure models was dictated by the positive effects of NAP/SAL in those studies, compared to the lack of effect in the rat and mouse postnatal exposure models. Dr. Goodlett will continue to collaborate on the new studies

testing the protective effects of NAP/SAL using behavioral analyses of spatial learning and locomotor activity.

2. Our pilot data indicate 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.

3. Our pilot study on facial dysmorphology in mice and the micro-CT analysis of craniofacial morphology will be a comparative study with that of human telemetric analysis of facial dysmorphology.

VIII. Plans for the next year:

1. In coordinating with Kathy Sulik, we will adopt new alcohol treatment paradigm which includes alcohol exposure prior and during the pregnancy.

2. Develop Micro-CT analysis of craniofacial dysmorphology including high throughput image acquirement and software testing.

3. Develop a 2-D to 3-D algorithm of facial imaging analysis for comparison with human facial dysmorphology.

IX. Publications:

Summary of previous funding and publications:

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 potential for use as a therapeutic agent to protect against alcohol-induced neuroteratogenesis. Data collected to date indicate that NAP and SAL are effective at the prenatal stages on fetal mouse brain growth (comparable to the stages of brain growth over the first trimester of human brain development). In contrast, the peptides did not protect against the effects of alcohol treatment in mice on postnatal day (PD) 7 or 7-9, nor in rats on PD 4-9 (periods in rodents comparable to stages of brain growth occurring over the human 3rd trimester). Furthermore, in a pilot study (Preliminary data 3 below), SAL and NAP were found to antagonize the facial or limb dysmorphogenesis produced by prenatal alcohol exposure in the mouse model.

Publications

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

2) Patel T, Zhou FC (2005) Ontogeny of 5-HT1A Receptor Expression in the Developing Hippocampus, Brain Res, Develop. Brain Res., 157(1): 42-57.

3) Goodlett CR, Horn KH, Zhou FC. (2005) Alcohol teratogenesis: mechanisms of damage and strategies for intervention. Exp Biol Med ;230(6):394-40.

4) Tran, T. D., Jackson, H. D., Horn, K. H. and Goodlett, C. R. (2005) Vitamin E does not protect against neonatal ethanol-induced cerebellar damage or deficits in eyeblink classical conditioning in rats. *Alcohol Clin Exp Res* 29, 117-29.

5) Zhou F, Yuan F, Goodlett C, Dysraphia and serotonin neuronal reduction induced by prenatal alcohol exposure is reduced by peptidergic agonist of activity dependent neurotrophic factor (submitted).

Publications related to proposed study

6) Zhou FC, Sari Y, Powrozek TA. Fetal alcohol exposure reduces serotonin innervation and compromises development of the forebrain along the serotonergic pathway. Alcohol Clin Exp Res. 29(2005)141-9

7) Powrozek TA and Zhou FC. Effects of prenatal alcohol exposure on the development of vibrissal somatosensory cortical barrel network. Dev. Brain Res., 155(2005)135-146.

8) Huang, J, Jain A, Fang A, Riley EP. Using Facial Images to Diagnose Fetal Alcohol Syndrome (FAS). IEEE

Proc. of Int'l Conf. on Information Technology: Coding and Computing (ITCC 2005). Vol. 1. New Trends in Image Processing. pp. 66-71, 2005.

X. Posters and Presentations:

Progress Report for Russia and Ukraine Clinical Project

I. Principal Investigator: Christina Chambers, Ph.D., M.P.H.

II. Title of Project: Spectrum of and Nutritional Risk Factors for FASD in Russia and Ukraine

III. Objectives:

The principal goals of this CIFASD clinical project are to more fully delineate the range of expression of alcohol-related birth outcomes in relation to timing and dose in a prospectively ascertained sample of pregnant alcohol-consuming women and their children, to examine the role of nutritional factors in alcohol-related birth outcomes, to test the effectiveness of a multi-micronutrient intervention with or without choline in reducing alcohol-related effects, and to evaluate various methods of earlier diagnosis of affected children.

The specific aims of this project are:

1. To measure the birth prevalence and range of alcohol-related physical features and neurobehavioral impairment among children born to women who report consuming moderate to heavy amounts of alcohol in pregnancy relative to children born to mothers who report consuming low amounts or no alcohol during pregnancy. Within this aim, we will:
    a. Evaluate alcohol quantity, frequency and timing in relation to growth, structure and neurobehavior
    b. Assess infant development using early infancy measures of processing speed and attentional regulation skills to measures from the BSID II
2. To evaluate the contribution of maternal nutritional status of specific micronutrients to risk for various features of FASD including growth deficiency, structural features, and neurobehavioral impairment. Within this aim, we will:
    a. Assess the relation of baseline nutritional status as measured in early pregnancy to alcohol exposure group
    b. Evaluate the impact of micronutrient supplementation with or without choline on change in nutritional status from baseline to third trimester in alcohol-exposed vs. comparison pregnancies
    c. Evaluate the predictive value of nutritional status for specific micronutrients with respect to alcohol exposure and specific pregnancy outcomes.
    d. Examine the relationship between indicators of oxidative stress and alcohol-related birth outcomes
3. To provide the performance site, local resources and human subjects for other current or proposed CIFASD projects.
    a. 2-D ultrasound imaging
    b. 3-D facial imaging

IV. Methods:

This CIFASD clinical project continues with recruitment at the previously identified locations in the Moscow Region of Russia, and adds a parallel site in Rivne, Ukraine – one of the two locations in Ukraine where a pilot project was completed in the previous round of CIFASD funding. Pregnant women are screened for alcohol consumption at prenatal care facilities and recruited at both sites on the basis of binge or frequent drinking in the month around conception and/or in the most recent month. Upon enrollment, at both sites, maternal interviews are used to collect additional information, blood samples are collected at enrollment and in the third trimester, and subjects are randomized to the multi-micronutrient with or without choline intervention.
Liveborn infants receive the standard CIFASD dysmorphological examination at either site, and all infants receive a neurobehavioral evaluation using the BSID II at 6 and 12 months of age. At the Ukraine site, additional prenatal ultrasound and fetal behavior measures have been incorporated as a Developmental Project (AD Hull, PI), and an additional neurobehavioral measure involving heart rate

monitoring to evaluate attentional regulation has been incorporated. In addition, at 6 months of age, 3-D facial imaging is planned (T Foroud, PI).

V. Accomplishments and Results:

- Since the notice of award, two trips have been made to Ukraine and to Russia by members of the study team to initiate the renewal protocols and renegotiate the specific aims following the 10% budget cut. Co-investigators Claire Coles, Julie Kable, Carl Keen and Ken Jones have visited Ukraine in September along with the PI and Ed Riley, and following an independent visit in early September by Andy Hull. Claire Coles, Carl Keen Ken Jones and the PI followed this with a visit to the Russian site in October/November.
- With co-investigators Claire Coles and Julie Kable, the data dictionary and input tool for the infant neurobehavioral assessments (BSID II and maternal questionnaire) have been finalized with the Informatics Core and the questionnaire has beentranslated.
- A secure intranet site has been established for exchange of data, photographs and documents, with password access established for investigators, data managers, and the Informatics Core staff.
- Equipment for the infant heart rate monitoring has been purchased, carried into Ukraine, assembled and tested by Dr. Kable, and training has been provided for Ukrainian staff on the use of this equipment; a plan for validation of testing protocols, and transmission of data has been established.
- Dr. Hull has developed a protocol for the additional ultrasound measures to be incorporated into the renewal project at the Ukraine site, and trained the Ukrainian ultrasound technicians on this protocol.
- Protocols for the administration of the vitamin supplements have been developed and the specific choline supplements selected for each site.
- With co-investigator, Lily Xu, a randomization protocol has been developed for assignment to the vitamin intervention in Ukraine.
- Revised human subjects protocols have been reviewed in the U.S. in response to pending IRB approval and are being simultaneously reviewed in the foreign sites. Biological materials transfer agreements for Rivne are being processed and the protocol for shipping established. We have met with CRDF staff in Moscow and initiated the renewal of their contract.
- Blood sampling and analytical protocols for the samples have been established by co-investigators Carl Keen and Jan Uriu-Adams; laboratory validation of the choline measurement technique has been performed in Dr. Keen's lab and the method outlined for use in this project both in the Russian laboratory and in Dr. Keen's lab. Because analysis of blood is being performed in Moscow for the Russian site and in the U.S. for the Ukrainian site, the Russian lab has selected a technician who is training in Dr. Keen's lab for the first two weeks in March, 2008, on the established technique for choline and metabolites analysis.
- Dr. Keen's group has taken the lead submitting a grant application for additional funding from the Allen Foundation to accomplish the exploratory aim regarding measures of oxidative stress.
- Analyses of data collected continues:
    - In collaboration with Ken Jones, Sarah Mattson and Claire Coles, hypotheses regarding the predictive value of specific alcohol-related structural features for IQ measures by child's age category, across selected CIFASD sites have been tested (see Table 1 below).
    - In collaboration with consultants Sharon Wilsnack and Arlinda Kristjanson, hypotheses regarding paternal drinking and perception of partner relationship quality vs. pregnancy outcome have been tested and are being compared to data from the Genesis project (see Table 2 below). Additional analyses regarding the correlation between drinking patterns reported on the screener vs. drinking reported on the first interview regarding the same periconceptional time period are being conducted.

- Analyses of the relation of selected measures of alcohol abuse and binge drinking with respect to the prevalence of alcohol-related physical features have been conducted (see Table 3 below).
- In collaboration with Andy Hull, final analyses of the prenatal ultrasound pilot data with respect to alcohol exposure have been completed, and a publication is in press with the *Journal of Ultrasound in Obstetrics and Gynecology* (see Table 4 below). Analyses regarding prenatal ultrasound measures and physical features of FASD have also been completed and a manuscript is in preparation.

Table 1. Predictors (physical features) of FSIQ among Younger and Older Children in the FAS or Deferred Groups (N=323)*

Growth measurements and structural features	Younger Children (≤ 6 years) N=83		Older Children (> 6 years) N=240	
	β	p-value	β	p-value
Weight ≤ 10thpercentile	-4.4	0.083	-10.5	<0.001
Height ≤ 10thpercentile	0.1	0.985	-12.8	<0.001
OFC ≤ 10thpercentile	-4.5	0.074	-15.2	<0.001
PFL ≤ 10thpercentile	-3.3	0.198	-6.3	0.009
Smooth philtrum	2.6	0.374	-1.8	0.490
Thin Vermilion Border	-0.1	0.981	-1.6	0.556
Campodactyly	-2.2	0.476	-3.3	0.207
Hockey stick crease	3.1	0.478	2.2	0.467
Other altered palmar creases	2.4	0.527	-1.5	0.689
Clinodactyly	3.5	0.324	-0.7	0.821
ICD ≤ 25th%	1.5	0.610	-4.6	0.164
Long Philtrum	-5.4	0.095	-3.0	0.235

*Using linear regression; FSIQ from Leiter; sample drawn from South Africa (May and Jacobson), Plain (May), San Diego (Mattson), Moscow (Mattson), Finland (Mattson)

Table 2. Maternal Knowledge, Relationship, and Influence of Paternal Alcohol Consumption as Predictors of Maternal Binge Drinking During Pregnancy (n=166).

Predictors	OR	95% CI	Overall p-value
Maternal Knowledge:			
Inaccurately reported that heavy drinking during pregnancy does not increase the chance of:			
Miscarriage	3.26	1.51; 7.04	0.003
Mental retardation of the child	1.55	0.56; 4.25	0.397
Low birth weight of the baby	2.56	1.28; 5.12	0.008
Birth defects	3.15	1.22; 8.11	0.017
Relationship:			
Degree of satisfaction in the relationship (from extremely unhappy to extremely happy)*	0.28	0.16; 0.49	<0.001
Quarrel (from quarrels with physical fights to solving disagreements without quarreling)**	0.61	0.42; 0.88	0.008
Paternal Drinking Habits:			
Frequency of alcohol consumption by the partner:			<0.0001
< once a week	1.0	--	
1-2 times a week	11.80	4.89; 28.44	
3-4 times a week	32.44	8.88; 118.59	
Every day / almost every day	12.17	1.79; 82.86	
Quantity of alcohol consumption per occasion by the partner:			<0.0001
1-2 drinks	1.0	--	
3-4 drinks	8.57	3.55; 20.71	
≥ 5 drinks	39.0	12.20; 124.72	
Paternal TWEAK			<0.0001
TWEAK <2	1.0	--	
TWEAK ≥ 2	27.73	7.92; 97.01	

* Likert scale (1-5). OR is presented for an increase in each point from extremely unhappy to extremely happy (being happy in the relationship is associated with a decreased risk of binge drinking)

** Likert scale (1-4). OR is presented for an increase for problem-solving skills (better problem-solving skills are associated with a decreased risk of binge drinking)

Table 3. Symptoms of Abuse and Binge Drinking vs. Structural Features of FASD

Measure	FAS (N=3)	FASD (N=18)	No Features (N=120)	p-value
	Mean±s.d	Mean±s.d	Mean±s.d	
12 months before Pregnancy Interview				
AUDIT score	8.3±1.5	6.0±5.2	2.3±3.4	<0.0001
TWEAK score	3.3±2.3	1.9±1.6	0.6±1.3	<0.0001
During Pregnancy				
Gestational age at the time of the interview (wks)	26.0±7.0	20.1±4.5	18.7±7.5	0.173
Largest # of drinks in 24h period during pregnancy	4.0±1.0	3.7±2.4	2.8±2.5	0.334
	%	%	%	
At least one binge episode (≥4 drinks)	66.7	50.0	7.5	<0.0001

Table 4. Fetal Brain Measures Assessed During the Second Trimester among Alcohol-Exposed and Control Subjects.

Measures:	**Exposed** (N=66)	**Controls** (N=64)	**p-value**[***]
	Adjusted[*] mean±s.e.	Adjusted[*] mean±s.e.	
Transverse Cerebellar Diameter (mm)	21.8±0.2	22.0±0.2	NS
Occipital Frontal Diameter (mm)	68.5±0.6	68.3±0.6	NS
Caval-Calvarial Distance (mm)	25.6±0.3	26.6±0.4	<0.05
Frontothalamic Distance (mm)	40.5±0.5	41.6±0.5	<0.05
Outer Orbital Diameter (mm)	36.1±0.3	35.9±9	NS
Interorbital Distance (mm)	11.0±0.2	11.3±0.2	NS
Orbital Diameter (mm)	11.5±0.2	11.6±0.2	NS

*Adjusted for gestational age and maternal smoking

VI. Discussion

Consistent with the aims of the Consortium and of this project, we are making progress on confirming that reported alcohol consumption is at least consistent with other measures of abuse, and is predictive of structural features of FASD. We are also making progress in demonstrating that the target features are being recognized in infancy by trained neonatologists/geneticists. This along with the promising initial data on prenatal ultrasound measures demonstrates progress toward accomplishment of the aims regarding measurement of the spectrum of alcohol-related effects in the context of varying alcohol consumption patterns and other risk factors, and the aim of improving earlier diagnosis. In addition, Drs. Cole and Kable have made progress in setting up the new infant testing equipment in Ukraine and training staff on the procedures.

Continuing concerns are the need to meet sample size requirements in both sites, the fact that blood measures are being conducted in two different laboratory settings, and that the cost of doing business in Moscow has increased dramatically over the last year, over and above the effects of the dollar exchange rate. We are addressing these concerns with additional training for the Moscow lab personnel, and by maintaining flexibility in our needs to meet sample size requirements with the possibility of extending efforts in Ukraine to supplement those in Russia.

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

In October, 2007, Dr. Keen met with the PASS steering committee to describe the nutritional component of the CIFASD clinical project for consideration as a possible parallel component of the PASS study. Drs. Mattson and Riley have worked with Drs. Jones, Chambers, Bakhireva, and Coles to develop hypotheses regarding change in physical features with age and the predictive value of these features for FSIQ as noted above. Drs. Chambers, Coles, Kable and Bakhireva have worked with the Informatics Core staff to develop and finalize the neurobehavioral tools. We anticipate that the next key comparisons will be regarding the baseline nutritional levels of choline and metabolites in relation to alcohol consumption in the human data vs. the animal models in collaboration with Drs. Thomas and Cudd.

VIII. Plans for the Next Year:

Recruitment will restart in Ukraine in February. In March, 2008, we plan to invite families previously involved in the pilot study to participate in neurobehavioral testing in Rivne, which will allow Drs. Kable and Coles to provide hands-on training experience, while also providing testing results on children for whom we have already collected exposure data, prenatal ultrasound measures, and physical exam data. We plan to do the first run of choline status measures in both sites within the next four months.

IX. Publications:

Kfir M, Yevtushok L, Onishchenko S, Wertelecki W, Bakhireva L, Chambers CD, Jones KL, Hull AD Can prenatal ultrasound detect the effects of in utero alcohol exposure? – A pilot study. In press. Ultrasound in Obstetrics and Gynecology.

X. Posters and Presentations:

M. Kfir, A. Hull, L. Yevtushok. S. Onishchenko, W. Wertelecki, L. Bakhireva, C. Chambers, K. Jones. Evaluation of Early Markers of Prenatal Alcohol Exposure. Platform presentation at the American Institute of Ultrasound in Medicine Annual Convention. March 15-18, 2007. New York, NY.

Jones KL, Hull AD, Bakhireva LN, Wertelecki WW, Yevtushok L, Sosynyuk Z, Shevchenko S, Zharko L, Onishenko S, Drapkina I, Chambers CD. Fetal Ultrasound Measures as Predictors of Alcohol-Related Physical Features in the Newborn: Preliminary Results. Birth Defects Research Part A: Clin and Molec Teratol 2007; 79:373.

Chambers CD. Measuring worldwide incidence of FASD. Birth Defects Research Part A: Clin and Molec Teratol 2007; 79:384.

Kfir M Platform presentation at the 12th World Congress of the International Society of Ultrasound in Obstetrics and Gynecology, November 2-7, 2007, New York, NY.

Chambers CD. Fetal Alcohol Spectrum Disorders in Russia and Ukraine - Diagnosis and Intervention/Prevention' Grand Rounds University of California, San Diego and Rady Childrens' Hospital, 12/10/2007

3 abstracts submitted to the Teratology Society - 2008

Progress Report for Facial Imaging

I. Principal Investigator: Tatiana Foroud, Ph.D. (PI), Elizabeth Moore, Ph.D. (Co-I), Richard Ward, Ph.D. (Co-I), Shiaofen Fang, Ph.D. (Co-I)

II. Title of Project: Facial Imaging Project

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, which produce the clinical features associated with FAS and thereby provide improved understanding of the pathophysiological effects of ethanol on human development.

IV. Methods:

The project is currently continuing to analyze data collected over the past 4 years and is also preparing to collect data using a new 3D imaging system. Previously, facial imaging data was collected from subjects seen in Buffalo, Finland, San Diego and South Africa. Dr. Elizabeth Moore has been 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 3-D 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 project investigators.

We will now utilize a new technology (3dMD system) which has several advantages over the system (Minolta Vivid910fw) used to capture 3D images in the past 4 years. First, the acquisition time is extremely rapid, only 1.5 milliseconds. This is similar to flash photography and eliminates blurred or distorted images from subject movement, which is common in young children. This is in contrast to our current acquisition time of 0.6 seconds. This dramatic decrease in acquisition time will allow us to image much younger children. The new system has less restrictive lighting conditions, allowing us greater flexibility for the site of data acquisition. Calibration is also much faster in this new system, only taking 5 minutes and only requiring two photos of a 3dMD provided calibration target. Finally, there is higher quality and more accurate color data with the new system. The 3dMD has 2.1 megapixel color cameras (1600x1200) while the Minolta has sub megapixel quality (640x480). In addition, the 3dMD color data does not suffer from poor lighting as did the previous Minolta system.

There are other advantages with regard to image processing. First, the new system includes automatic merging of the camera data to create the three dimensional image. This is possible due to the dual camera positioning which captures ‘ear-to-ear’ data, often referred to as a continuous point cloud. Previously, all merging (or stitching) was performed by a member of the core and was extremely time consuming. There was also the risk of lost data due to changes in facial expression between frontal and lateral shots. There is also greater geometric accuracy in the new system. The 3dMD system has an accuracy of about 0.5 mm XYZ, while the previous system had about 0.9mm X/Y and 0.3 mm Z accuracy. With the new system, we anticipate higher data quality.

Our current focus is developing the final protocols for this new system, performing a small reliability and inter-camera study and preparing the sites for the collection of data using this new system.

V. Accomplishments and Results:

1. Specific Aim 1: Train and supervise personnel at each recruitment site to ensure collection of standardized data. Training was successfully performed for all site personnel in Buffalo, Finland, San Diego and South Africa. The approach that was successful in the training of sites for the Minolta Vivid910fw will be adopted for training sites in the use of the new 3dMD system.

2. Specific Aim 2: Analyze the 3-D facial imaging data to identify the measurements that most efficiently differentiate alcohol exposed from control subjects. A total of 276 subjects were recruited from four sites (South Africa, Finland, Buffalo, and San Diego). Computerized anthropometry was employed to identify facial features that could distinguish FAS patients from controls across a wide age range and across ethnically disparate study populations. Subjects were placed into one of four populations based on their ancestry (Cape Coloured, Finnish Caucasian, African American, or North American Caucasian). Analyses performed in each of the four study populations were able to identify a unique set of variables which provided excellent discrimination between the two groups (FAS, control). In each study group, at least one ocular-related measurement, shortened palpebral fissure, reduced outer canthal width, or reduced inner canthal width, was included in the final classification model. We found measurements that reflected reduced size of the eye orbit to be a consistent feature discriminating FAS and controls across each study population. However, each population had a unique, though often overlapping, set of variables which discriminated the two groups, suggesting important ethnic differences in the presentation of FAS. It is possible that these differences were accentuated by the wide age distribution of the study subjects. These results were recently published (Moore et al, 2007).

3. 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. 3-D facial images from 149 individuals (86 FAS; 63 Control) recruited from two study sites (South Africa and Finland) were analyzed using computer graphics, machine learning and pattern recognition techniques to automatically identify a set of facial features that best discriminated individuals with FAS from controls in each sample. An automated feature detection and analysis technique was developed and applied to the two study populations. A unique set of facial regions and features was identified for each population that accurately discriminated FAS and control faces without any human intervention. Our results demonstrate that computer algorithms can be used to automatically detect facial features that can discriminate FAS and control faces. These results were recently submitted to Orthodontics and Craniofacial Research (Fang et al, submitted).

   We have recently developed a new visual approach to 3D feature detection of facial images for shape classification. Using 3-D shading and visualization techniques in computer graphics and modern graphics hardware, we were able to provide an interactive system that allowed the users to interactively explore the differences of the geometric properties of the facial surfaces between two groups of data, and detect facial regions that were the most discriminatory. This technique can be used as a guide for feature selection in quantitative feature analysis (such as machine learning and pattern recognition) for determining the diagnostic functions. It is particularly useful for small sample problems where traditional feature selection methods may not be effective. Unlike previous feature visualization techniques in which features were extracted before the visualization, our approach used visualization as a mean of discovering and detecting new features. This approach was tested in the South African dataset and the results are currently being prepared for publication(McLaughlin et al., submitted).

4. 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 been successful in training remote sites in the collection of 3D facial images. The methods used previously will be applied again as we train sites in the use of the 3dMD system. The focus in the new data collection will be longitudinal data collection, with a concentration on determining whether age or ethnicity or both, contribute to the differences recently observed in the features that distinguish FAS from controls.

VII. Interrelation with Aims of the Consortium and other projects:

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:

The focus in the coming year will be implementing the new 3dMD system at the study sites. In the short term, a pilot study will be completed at Indiana University using about 20 normal controls. 3-D facial images will be collected using both systems in all subjects. This will allow us to finalize the protocol for the new 3dMD system, will allow us to determine how best to utilize longitudinal data collected using different camera systems and will also provide interindividual and intercamera variability estimates.

IX. Publications:

Rogers J, Wernert E, Moore E, Ward R, Wetherill, LF, Foroud T. A multinational deployment of 3D laser scanning to study craniofacial dysmorphology in fetal alcohol spectrum disorders. In J. A. Beraldin, F. Remondino, & M. R. Shortis (Eds.). Proceedings of SPIE-IS&T Electronic Imaging, SPIE Vol. 6491, *Videometrics IX*. The International Society for Optical Engineering, Bellingham, Washington, 2007.

Moore ES, Ward RE, Wetherill LF, Rogers JL, Autti-Ramo I, Fagerlund A, Jacobson SW, Robinson LK, Hoyme HE, Mattson SN, Foroud T, and the CIFASD. Unique facial features distinguish fetal alcohol syndrome patients and controls in diverse ethnic populations. Alcoholism: Clinical and Experimental Research 31: 1707-1713, 2007.

Fang S, McLaughlin J, Fang J, Huang J, Autti-Ramo I, Fagerlund A, Jacobson SW, Robinson LK, Hoyme HE, Mattson SN, Riley E, Zhou F, Ward R, Moore ES, Foroud T, and the CIFASD. Automated diagnosis of fetal alcohol syndrome using 3D facial image analysis. Orthodontics and Craniofacial Research, submitted.

McLaughlin J, Fang S, Huang J, Jacobson SW, Hoyme HE, Robinson LK, Foroud T, and the CIFASD. Interactive Feature Visualization and Dectection for 3D Face Classification. Visual Computer, submitted.

X. Posters and Presentations:

Mattson S, Flury-Wetherill L, Foroud T, Rogers J, Moore E, Autti-Rämö I and the CIFASD Consortium. Classification of fetal alcohol syndrome using 3-D facial imaging and neuropsychological data. (Abstract), Alcoholism: Clinical and Experimental Research 31(suppl)(6):246A, 2007.

Ward RE, Moore ES, Wetherill L, Foroud T, and the Collaborative Initiative on Fetal Alcohol Spectrum Disorders Consortium. Population specific craniofacial variation and its relevance to the clinical application of anthropometry. American Association of Physical Anthropologists, submitted.

Moore ES. The use of three-dimensional facial images to identify FASD across ethnic groups. Oral presentation at The 2nd International Conference on Fetal Alcohol Spectrum Disorder, Victoria, British Columbia, March 2007.

Ward RE. Screening Children for fetal alcohol spectrum disorders using 3-D Laser Photography. Oral presentation to the Midwest Association of Toxicology and Therapeutic Drug Monitoring meeting, April 25 2007.

Progress Report for a Multisite Neurobehavioral Assessment of FASD

I. Principal Investigator: Sarah N. Mattson, Ph.D.
Key Personnel: Colleen Adnams, MD, Co-PI, subcontract
Claire D. Coles, Ph.D., PI, subcontract
Julie A. Kable, Ph.D., Co-I, subcontract
Wendy Kalberg, Ph.D., Co-PI, subcontract
Philip A. May, Ph.D., PI, subcontract
Edward P. Riley, Ph.D., Co-PI
Elizabeth R. Sowell, Ph.D., PI, subcontract

II. Title of Project: A Multisite Neurobehavioral Assessment of FASD

III. Objectives:

The primary aim of this project is to determine whether a neurobehavioral phenotype exists in children with fetal alcohol syndrome, whether the same phenotype exists in children with FASD who lack facial dysmorphology, and whether the phenotype can be used for differential diagnosis. Secondary aims, involving collaboration with other CIFASD projects and cores, are to determine the relationship between brain dysmorphology, facial dysmorphology, and neurobehavioral function.

IV. Methods:

A standard neurobehavioral protocol will be administered to four groups of children at six sites and will address the functional domains of executive function, working memory, verbal function, and psychological symptomatology. In addition to children with FASD and non-exposed controls, children with low IQ scores or ADHD will be included as contrast samples. Using this heterogeneous sample and multivariate statistical methods,a neurobehavioral profile specific to FASD will be sought. In addition, participants will be assessed using methodology prescribed by the Dysmorphology Core and the facial and brain imaging projects of the CIFASD. Data from three broad domains (neurobehavior, dysmorphology, and brain morphology and function) will be analyzed both separately and together to address the main aim of the CIFASD: improving the diagnostic criteria for FASD.

The current project assumed responsibility for the functions previously carried out by the Neurobehavioral Core. As such, the main site (San Diego) is now responsible for preparing all testing materials including purchasing tests and test materials, and creating and distributing administration manuals, summary forms, reliability protocols and forms, and scoring manuals. Training sessions are to be conducted at the San Diego site during the first year with follow-up sessions each year for reliability purposes, either in San Diego or in conjunction with one of the annual PI meetings. In addition, we are responsible for collaborating with the Informatics Core (C. Stewart, PI) on creating the data dictionary for the input tool for the current data and on pilot testing of the input and upload tools created for this project.

Six sites are used for participant recruitment. Three of these sites were included in the last funding period for the CIFASD through 2 separate projects. The current sites are: (1) Center for Behavioral Teratology, San Diego State University, San Diego, CA; (2) Marcus Institute, a Division of Kennedy-Krieger Institute at Emory University, Atlanta, GA; (3) University of New Mexico, Albuquerque, NM; (4) Seven Northern Plains communities, including six Indian reservations; (5) University of Cape Town, South Africa, and a town in the Western Cape Province with the highest recorded prevalence of FAS in the world; and (5) the University of California, Los Angeles, Los Angeles, CA. The combination of these sites allows for a study population that is both large in number and heterogeneous in nature. Such a sample will ensure our results are both accurate and unbiased by specific site characteristics.

V. Accomplishments and Results:

1. *Previous Award.* The progress report from the renewal application, which details our progress during the previous funding period is included in Appendix 1. Briefly, two studies are in manuscript phase, including analysis of the virtual water maze task and the latent profile analysis of the CIFASD

neurobehavioral data. The manuscript for the watermaze study was not accepted by *Neuropsychologia* and we are reanalyzing and revising the manuscript for another journal. The latent profile analysis was presented at RSA in 2007 and we are preparing the manuscript for publication. We have also published three papers documenting results from imaging studies, and these are detailed in the appendix.

2. *Current Award.* Funding began on September 30, 2007 and significant progress has been achieved as follows:

    1. General Progress (San Diego).

        a. *IRB Approvals*. We submitted applications for IRB approval for the neuropsychological, dysmorphology, brain imaging, and 3D imaging portions of the study at each site. Approvals were granted at the SDSU site and are pending at all other sites.

        b. *Subcontracts*. Two of the three subcontracts have been submitted to the sites and we are awaiting their return. The third subcontract, to the Marcus Institute, is ready to submit but we have not received critical information back from institute personnel. Once we receive this, we will submit the subcontract paperwork to Marcus. Each subcontract has a clause regarding IRB approval. Funds related to data collection cannot be released until site-specific IRB approvals are in place.

        c. *Hiring*. We have in place staff necessary to conduct the study, including a psychometrist, recruiter, and research associates/assistants. The subcontract sites have personnel in place, with the exception of Atlanta and South Africa which are waiting for funds to be released to hire personnel.

        d. *Purchasing*. We have purchased materials necessary to begin data collection and have ordered the CANTAB computers for each site. We are hoping that they will be here by the time we have our second training meeting, see below.

        e. *Material Development*. We have created working drafts of our test administration materials and scoring materials. These will be finalized in January prior to the training meeting, see below.

        f. *Training*. We conducted our first training meeting in San Diego on December 3-5, 2007. In attendance were data collection staff from University of New Mexico and UCLA. Participants were trained on data collection procedures and received materials for pilot testing and instructions for reliability procedures. They are in the process of practicing administration and will submit the reliability materials prior to the second training meeting, which is to be held January 14-15, 2008 in San Diego. Site staff from South Africa and Atlanta will also be in attendance during the second training and will submit reliability tapes after that. The goal of training is to ensure that all sites are collecting data in the same manner, and are reliable with us and with each other. The second training will cover scoring procedures as well as procedures pertaining to the input and upload tools.

        g. *Database Development*. We have been working with the Informatics Core to develop the input tool for the new neurobehavioral test battery (CIFASD Neurobehavioral Phase II). We have received and piloted the beta version of this input tool and are actively working with them to move it towards its final form.

    2. Site-Specific Progress: All sites have submitted IRB applications and are awaiting approval. In some cases, modifications have already been requested and are pending. Only SDSU has final IRB approval at this point, but we anticipate approval at all sites shortly. Two sites attended the first training meeting in December 2007 and all sites will be present at the second training in January 2008. Our budget allows data collection to begin in month 7 (April 2008) and we are hoping to begin data collection earlier (March 2008) at some of the sites.

3. Neurobehavioral Testing. Data collection has not begun, see above. We are continuing to collect data from a small number of subjects using the Phase I test battery. To date we have tested 105 children using this test battery. Some of these data have been analyzed and 3 studies have either been published or presented as abstracts in the last year and are listed in the publication list, below.
4. MRI Evaluations: The San Diego site is also involved in the brain imaging project (E. Sowell, PI). We have IRB approval for this component and are ready to begin data collection approximately at the same time as the neurobehavioral data collection. We also collected MRI data from 42 subjects at the San Diego site during the CIFASD Phase I funding period. Some of these data have been analyzed and 7 studies have either been published or presented as abstracts in the last year and are listed in the publication list, below.
5. 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 100 children and transferred these data to Elizabeth Moore. Some of these data have been analyzed and 1 study has either been published or presented as abstracts and is listed in the publication list, below. An additional abstract presented at the 2008 RSA meeting included data from both the neurobehavior and facial imaging projects.
6. Dysmorphology: Since the beginning of the CIFASD Phase I, 99 children have been examined by the Dysmorphology Core.

VI. Discussion:

Although we have not begun data collection, we have made substantial progress since the beginning of the funding period as described above. We are poised to begin data collection in two domains: neurobehavior and brain imaging and continue data collection in two domains: 3D facial imaging and dysmorphology. Data collection will be conducted at multiple sites with varied subject characteristics. We will thus be in the position to have access to a large, heterogeneous population on which to test our hypotheses.

VII. Interrelation with Aims of the Consortium and other projects:

This project relates to the overall aim of the consortium project 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.

VIII. Plans for the next year:

During the next year, we plan to initiate data collection at all 6 sites using 3D photo, dysmorphology, brain imaging, and neurobehavioral testing. We will finalize our administration and scoring, and reliability procedures and will continue to provide administrative support to the sites involved in neurobehavioral testing. We will continue to work with the Informatics Core as well as the 3D facial imaging, brain imaging, and dysmorphology projects.

IX. Publications:

Mattson, S.N., Riley, E.P., Autti-Rämö, I., May, P.A., Konovalova, V., Jones, K.L., Roesch, S.C., and the CIFASD. Spatial learning and navigation deficits in an international sample of children with heavy prenatal alcohol exposure. Manuscript under revision after review.

Sowell, E.R, Mattson, S.N., Kan, E., Thompson, P.M., Riley, E.P., and Toga, A.W. (2008). Abnormal cortical thickness and brain-behavior correlation patterns in individuals with heavy prenatal alcohol exposure. Cerebral Cortex 18 (1), 136-144. doi:10.1093/cercor/bhm039 http://cercor.oxfordjournals.org/cgi/content/full/18/1/136

Moore, E.S., Ward, R.E., Flury Wetherill, L., Rogers, J.L., Autti-Rämö, I., Fagerlund, Å., Jacobson, S.W., Robinson, L.K., Hoyme, H.E., Mattson, S.N., Foroud, T., and the CIFASD. (2007). Unique facial features distinguish fetal alcohol syndrome patients and controls in diverse ethnic populations. Alcoholism: Clinical and Experimental Research, 31 (10), 1707-1731.

Fryer, S.L., Tapert, S.F., Mattson, S.N., Paulus, M.P., Spadoni, A.D., and Riley, E.P. (2007). Prenatal alcohol exposure affects frontal-striatal BOLD response during inhibitory control. Alcoholism: Clinical and Experimental Research, 31 (8), 1415-1424.

Sowell, E.R., Lu, L.H., O'Hare, E.D., McCourt, S.T., Mattson, S.N., O'Connor, M.J., and Bookheimer, S.Y. (2007). Functional magnetic resonance imaging of verbal learning in children with heavy prenatal alcohol exposure. NeuroReport, 18 (7), 635-639.

X. Posters and Presentations:

Mattson, S.N., Fryer, S.L., Spadoni, A., Bazinet, A., Sowell, E.R., and Riley, E.P. (2008). Functional brain changes in children and adolescents with heavy prenatal alcohol exposure. To be presented at the International Neuropsychological Society meeting, Waikaloa, February 2008.

O'Hare, E.D., Lu, L.H., Bookheimer, S.Y., McCourt, S.T., Houston, S.M., Mattson, S.N., O'Connor, M.J., and Sowell, E.R. (2007). Increased dorsal frontal and inferior parietal activation during verbal working memory among children and adolescents with prenatal alcohol exposure. 37th Annual Meeting, Society for Neuroscience, San Diego, November 3-7, 2007. Program No. 610.15. Online Abstract Viewer.

Mattson, S.N., Roesch, S.C., Riley, E.P., Adnams, C., Autti-Rämö, I., Fagerlund, Å., Kalberg, W., Korkman, M., May, P.A., and the CIFASD. (2007). Neurobehavioral profile of children with heavy prenatal alcohol exposure. Presented at the Research Society on Alcoholism meeting, Chicago, July 2007. Alcoholism: Clinical and Experimental Research, 31 (6), 185A.

Mattson, S.N., Flury-Wetherill, L., Foroud, T., Rogers, J., Ward, R., Moore, E., Autti-Rämö, I., Korkman, M., Fagerlund, Å., Riley, E.P., and the CIFASD (2007). Classification of fetal alcohol syndrome using combined 3-D facial imaging and neuropsychological data. Presented at the Research Society on Alcoholism meeting, Chicago, July 2007. Alcoholism: Clinical and Experimental Research, 31 (6), 246A.

McGee, C.L., Vaurio, L., Riley, E.P., and Mattson, S.N. (2007). Comparison of multiple measures of intelligence in children with heavy prenatal alcohol exposure and non-exposed controls. Presented at the Research Society on Alcoholism meeting, Chicago, July 2007. Alcoholism: Clinical and Experimental Research, 31 (6), 106A.

Fryer, S.L., Noonan, S.K., Tapert, S.F., Mattson, S.N., Spadoni, A.D., and Riley, E.P. (2007). Examination of functional connectivity during response inhibition task performance in individuals with prenatal alcohol exposure. Presented at the Research Society on Alcoholism meeting, Chicago, July 2007. Alcoholism: Clinical and Experimental Research, 31 (6), 68A.

Spadoni, A.D., Bazinet, A.D., Fryer, S.L., Tapert, S.F., Mattson, S.N., and Riley, E.P. (2007). Prenatal alcohol exposure and spatial working memory in adolescents and pre-adolescents: An fMRI study. Presented at the Research Society on Alcoholism meeting, Chicago, July 2007. Alcoholism: Clinical and Experimental Research, 31 (6), 106A.

Arenson, A.D., Bakhireva, L., Chambers, T., Deximo, C., Foroud, T., Jacobson, J., Jacobson, S., Jones, K.L., Mattson, S.N., May, P., Moore, E., Ogle, K., Riley, E., Robinson, L., Rogers, J., Streissguth, A., Tavares, M., Urbanski, J., Yezerets, H., and Stewart, C.A. (2007). Implementation of a distributed architecture for managing collection and dissemination of data for fetal alcohol spectrum disorders research. Presented at the International Workshop on Distributed, High Performance and Grid Computing in Computational Biology (GCCB 2006). Lecture Notes in Bioinformatics (March 2007)

We are planning on submitting an abstract to the 2008 RSA meeting (due January 11, 2008), but it is not yet complete. It is an analysis of data collected through CIFASD Phase I and data collected at San Diego through other funding sources.

Progress Report from Dr. Phil May, PI of UNM/SA Sites

CIFASD Neurobehavioral Project – Progress Report

Subcontract: Philip A. May, Ph.D.
The University of New Mexico/CASAA

Key Personnel: Wendy Kalberg, M.A., CED

Alfredo Aragon, Ph.D.
David Buckley, M.S.

We have spent the last few months working on the Institutional Review Board (IRB) packets for submission to the University of New Mexico and Indian Health Services (HIS) IRBs. It was necessary to go back to our selected American Indian sites to gain approval for this work through tribal resolutions. Those tribal resolutions were received the week of December 10th which allowed submission of the IRB packets to The University of New Mexico and Indian Health Services IRBs in both Billings Area (MT) and Aberdeen Area (SD, ND). We are awaiting review of those protocols, and anticipate feedback in January.

Alfredo Aragon, Ph.D., attended the first training held in San Diego for those who will test the children and oversee the data collection. Dr. Aragon will pilot test the battery with a child on December 19, 2007 at the Albuquerque office. He is also scheduled to attend the second training session in San Diego the week of January 14, 2008.

Finally, we have participated regularly in the Neurobehavioral conference calls that are scheduled to discuss the ongoing development and implementation of this project.

Progress Report from Dr. Claire Coles, PI of Atlanta Site

Marcus Subcontract Progress Report as of December 19, 2007

Since the project was approved, we have done the following in pursue of the Aims of the project:

1) IRB approval is required before any funds can be released. For this reason, we continue to have no staff or salary support. Based on the materials provided by Dr. Mattson, Dr. Coles completed IRB submission materials and submitted these to the Emory University School of Medicine Internal Review Board. This was done on September 19, 2007. The protocol qualified for expedited review. The IRB committee meeting was November 24, 2007. We await notice of the results.

2) Staff. The project will require a half-time project coordinator to manage subcontract activities and help in recruiting subjects from the Clinic clients. In addition, we will require a tester who will be trained in the study protocol. We have identified a suitable tester who will be available beginning February 2008. We have opened a position at Marcus for the project coordinator and advertised the position. Dr. Coles is currently reviewing applications and interviewing for this position. However, we will not be able to hire anyone until funds are available.

3) Administrators at San Diego State have communicated with Farah Chapes, the administrator at Marcus regarding the subcontract. Ms. Chapes has not yet responded.

4) Dr. Kable has communicated with Dr. Mattson and her staff in San Diego regarding site administration and testing protocols. Dr. Kable has arranged to go to San Diego for training on January 12-14, 2008.

Progress Report from Dr. Elizabeth Sowell, PI of UCLA Site

CIFASD Neurobehavioral Project – Progress Report
12/21/2007

Subcontract: Elizabeth Sowell, Ph.D.
University of California, Los Angeles

Progress for the renewal period relevant to this project, has been primarily in 2 areas, 1) IRB applications/approvals, and 2) training for the neurobehavioral testing battery.

1) In order to accommodate this new phase of the CIFASD, IRB applications were initiated in July of 2007 at which time we included the new fund number, and added Ken Jones as an investigator. In October of 2007, we asked permission to include an ADHD population, increase our neuropsychological testing time by 4 hours, increase scanning time by 30 minutes, increase testing time for parents to 3 hours, requested approval to deposit data into the CIFASD repository, requested approval to contact participants teachers to fill out questionnaires, and to include Sarah Mattson's grant number to accommodate the subcontract. Our IRB responded to these requests on December 4th requesting some minor clarifications, to which we responded on December 13. We expect to have full approval early in January.

2) Data acquisition staff from UCLA, along with staff from the University of New Mexico and San Diego State University, attended a training workshop in San Diego between December 3-5, 2007. General procedures for the neurobehavioral study were reviewed, including reliability, testing, and database procedures. The remainder of the workshop focused on administration of specific neuropsychological and behavioral measures, including the WISC-IV Integrated, Delis-Kaplan Executive Function System (D-KEFS), Leiter-R, Cambridge Neuropsychological Test Automated Battery (CANTAB), Behavior Rating Inventory of Executive Function (BRIEF), ASEBA Child Behavior Checklist (CBCL) and Teacher Report Form (TRF), Vineland-2, and Diagnostic Interview Schedule for Children (DISC-IV). A second training workshop is scheduled for January 14-15, 2008 in San Diego, and staff will be trained on scoring of measures, abstracting of subjects' history, and finalized procedures for data entry into the central repository.

Progress Report for Mapping the Brain, Face, and Neurocognitive Function in FASD

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

II. Title of Project: Mapping the Brain, the Face and Neurocognitive Function in FASD. (U01); U01 AA017122.

III. Objectives:

The Specific Aims are as follows:

1. To evaluate cross-sectionally and longitudinally the effects of prenatal alcohol exposure on brain morphology and function. We will study differences in the patterns of results that occur across populations where drinking patterns may vary by making FASD/control comparisons within sites, and comparing the results across sites.
2. To evaluate relationships between brain dysmorphology and facial dysmorphology both cross-sectionally and longitudinally to improve diagnostic criteria using facial morphology data from the dysmorphology core (cross-sectional data only) and the 3D camera project.
3. To determine whether the anatomical "phenotype" relates to neurobehavioral profiles in children with fetal alcohol syndrome or FASDs.
4. To investigate dysmorphology in the brains of human children based on findings in the mouse and sheep models conducted in the laboratories of Drs. Sulik, Zhou and Cudd.

IV. Methods:

To conduct brain imaging, neuropsychological evaluations, and 3D facial imaging.

V. Accomplishments and Results:

1. Progress Continued from the Last Funding Period (from the U24 Brain Imaging Core): Since our last progress report included in the renewal application, we have continued to analyze data collected by the brain imaging core. One paper that was In Press has now been published, one that was submitted has been published, and we have an entirely new manuscript on white matter abnormalities and neurobehavioral correlates that is now In Press in the *Journal of Neuroscience* (see Manuscripts Published section below). One abstract was published, and we continue to analyze data on a daily basis. Below, we will describe results from our newest study to illustrate the types of work we continue to do while we prepare for new data collection during the renewal period.

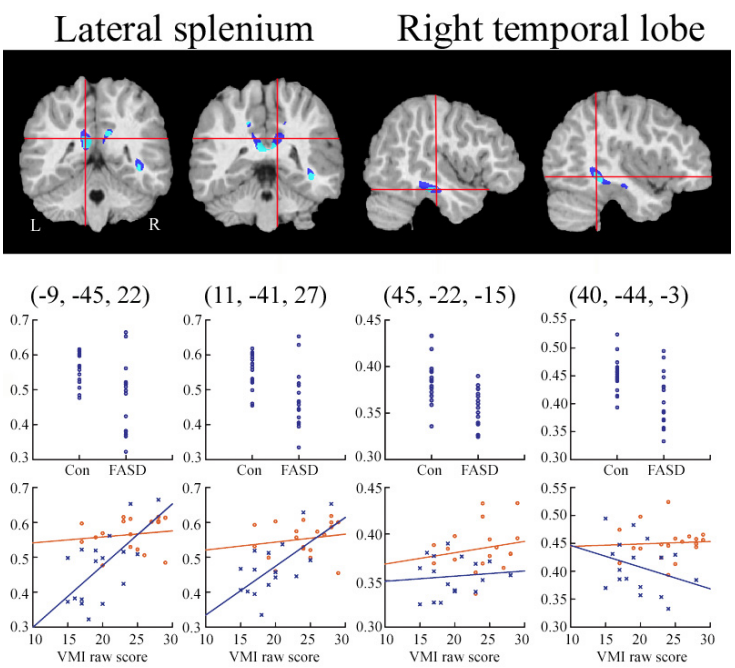


Figure 1: Region of interest (ROI) FA analyses and Brain Behavior Correlations. Coronal and sagittal slices of one representative subject's brain with significant clusters from the FA and WMD analyses. Shown in dark blue are regions where FA (but not WMD) was significantly lower in the individuals with FASDs relative to control subjects ($p < 0.05$, FDR correction for multiple comparisons). Regions in light blue are regions where WMD was lower in the FASD relative to controls ($p < 0.001$, uncorrected) and overlapped with regions of lower FA in FASD. The Y-axes for all graphs have FA values. In the top row of graphs, FA values for each subject are shown for the FASD and control groups. FA values were taken from ROIs (localized approximately at the intersection of the crosshairs in each image) of all voxels within an 8mm sphere in the unsmoothed, white matter masked FA volume for each individual. In the bottom row, FA values from the same ROIs are shown in relation to subjects' behavioral scores on the VMI. Control subjects are shown in red and FASD subjects in blue. Correlations between visuomotor integration (VMI) scores and FA were highly significant in the left and right lateral splenium ROIs within the FASD, but not control subjects, and not for either group in the anterior and posterior temporal ROIs.

Mapping White Matter Integrity and Neurobehavioral Correlates in FASD (In Press, *Journal of Neuroscience*): Brain structural abnormalities and neurocognitive dysfunction have been observed in individuals with fetal alcohol spectrum disorders (FASDs). Little is known about how white matter integrity is related to these functional and morphological deficits. We used a combination of diffusion tensor and T1-weighted magnetic resonance imaging to evaluate white matter integrity in individuals with FASDs and related these findings to neurocognitive deficits. Seventeen children and adolescents with FASDs were compared to 19 typically developing age and gender matched controls. Lower fractional anisotropy (FA) was observed in individuals with FASDs relative to controls in the right lateral temporal lobe and bilaterally in the lateral aspects of the splenium of the corpus callosum (see Figure 1). White matter density (WMD) was also lower in some, but not all regions where FA was lower. FA abnormalities were confirmed to be in areas of white matter in post hoc region of interest analyses, further supporting that less myelin or disorganized fiber tracts are associated with heavy prenatal alcohol exposure. Significant correlations between performance on a test of visuomotor integration and FA in bilateral splenium, but not temporal regions were observed within the FASD group. Correlations between the visuomotor task and FA within the splenium were not significant within the control group, and were not significant for measures of reading ability. This suggests that this region of white matter is particularly susceptible to damage from prenatal alcohol exposure and that disruption of splenial fibers in this group is associated with poorer visuomotor integration.

2. Progress for the Renewal Period: Progress for the renewal period has been primarily in 3 areas, 1) IRB applications/approvals, 2) planning image acquisition protocols to accommodate all 3 data collection sites, and 3) training for the neurobehavioral testing battery in collaboration with Sarah Mattson.

   In order to accommodate this new phase of the CIFASD, IRB applications were initiated in July of 2007 at which time we included the new fund number, and added Ken Jones as an investigator. In October of 2007, we asked permission to include an ADHD population, increase our neuropsychological testing time by 4 hours, increase scanning time by 30 minutes, increase testing time for parents to 3 hours, requested approval to deposit data into the CIFASD repository, requested approval to contact participants teachers to fill out questionnaires, and to include Sarah Mattson's grant number to accommodate the subcontract. Our

IRB responded to these requests on December 4th requesting some minor clarifications, to which we responded on December 13. We expect to have full approval early in January.

We have started the process of image acquisition planning with Drs. Sowell and Narr working closely on this project. Pilot data for the structural and functional images will be collected from UCLA's 3T Siemen's Allegra system (same as the system set up in South Africa) on January 6. Dr. Narr will serve as the "human phantom" for these purposes. Dr. Narr's first trip to South Africa is planned for February/March 2008. The goal for this trip is to initiate interface between South African investigators and imaging technical staff. Pilot studies are planned to collect data from Dr. Narr as the "human phantom," as well as regular phantom data. Given the considerable effort Dr. Narr is investing during these early stages, we have increased Dr. Narr's effort on the project from 2% (which was initially planned to cover only the time during which she was traveling to South Africa) to 8%. It is anticipated that her effort will reduce back to travel time only in subsequent years.

Data acquisition staff from UCLA, along with staff from the University of New Mexico and San Diego State University, attended a training workshop in San Diego between December 3-5, 2007. General procedures for the neurobehavioral study were reviewed, including reliability, testing, and database procedures. The remainder of the workshop focused on administration of specific neuropsychological and behavioral measures, including the WISC-IV Integrated, Delis-Kaplan Executive Function System (D-KEFS), Leiter-R, Cambridge Neuropsychological Test Automated Battery (CANTAB), Behavior Rating Inventory of Executive Function (BRIEF), ASEBA Child Behavior Checklist (CBCL) and Teacher Report Form (TRF), Vineland-2, and Diagnostic Interview Schedule for Children (DISC-IV). A second training workshop is scheduled for January 14-15, 2008 in San Diego, and staff will be trained on scoring of measures, abstracting of subjects' history, and finalized procedures for data entry into the central repository.

VI. Discussion:

VII. Interrelation with Aims of the Consortium and other projects:

Thus far, our integration with other projects has been limited to the neurobehavioral project as described above. Integration with the dysmorphology and facial imaging projects are anticipated as we get closer to recruitment scheduled to start during the first three months of 2008.

VIII. Plans for the next year:

We will continue to plan data acquisition, and data collection will begin.

IX. Publications:

Sowell ER, Mattson SN, Kan E, Thompson PM, Riley EP, Toga AW (2007a) Abnormal Cortical Thickness and Brain-Behavior Correlation Patterns in Individuals with Heavy Prenatal Alcohol Exposure. *Cereb Cortex* epub ahead of print.

Sowell ER, Lu LH, O'Hare ED, McCourt S, Mattson SN, O'Connor MJ, Bookheimer SY (2007b) Functional magnetic resonance imaging of verbal learning in children with heavy prenatal alcohol exposure. *NeuroReport* 18:635-639.

Sowell E.R., Johnson A., Kan E., Lu, L.H., Van Horn, J.D., Toga, A.W., O'Connor, M.J., and Bookheimer S.Y., (In Press) Mapping White Matter Integrity and Neurobehavioral Correlates in Children with Fetal Alcohol Spectrum Disorders. *Journal of Neuroscience*.

X. Posters and Presentations:

O'Hare, ED, Lu, LH, McCourt, ST, Bookheimer, SY, and Sowell, ER. Evidence for Frontal-Cerebellar Involvement in Verbal Working Memory in Typically Developing Children and Adolescents. 13th Annual Meeting of the Organization for Human Brain Mapping, Chicago, IL, USA. *NeuroImage Abstracts*, 2007.

Publications

Arenson, A. D., Bakhireva, L., Chambers, T., Deximo, C., Foroud, T., Jacobson, J., et al. (2007). Implementation of a distributed architecture for managing collection and dissemination of data for fetal alcohol spectrum disorders research. In A. Hofmann (Series Ed.) & W. Dubitzky, A. Schuster, P. Sloot, M. Schroeder, & M. Romberg (Vol. Eds.), *Lecture notes in computer science: Vol. 4360. Distributed, high-performance and grid computing in computational biology* (pp. 33-44). Heidelberg: Springer Berlin.

Arevalo, E., Shanmugasundararaj, S., Wilkemeyer, F., Dou, X., Chen, S., & Charness, M. E., et al. (2008). An alcohol binding site on the neural cell adhesion molecule L1. *Proceedings of the National Academy of Sciences of the United States of America, 105*, 371-375.

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Huang, J., Jain, A., Fang, A., & Riley, E. P. Using facial images to diagnose fetal alcohol syndrome (FAS). (2005). *International Conference on Information Technology: Coding and Computing, 2,* 66-71.

Jones KL, Hull AD, Bakhireva LN, Wertelecki WW, Yevtushok L, Sosynyuk Z, Shevchenko S, Zharko L, Onishenko S, Drapkina I, Chambers CD. Fetal Ultrasound Measures as Predictors of Alcohol-Related Physical Features in the Newborn: Preliminary Results. (2007) *Birth Defects Research Part A: Clin and Molec Teratol .79:373*

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Moore, E. S., Ward, R. E., Flury-Wetherill, L., Rogers, J. L., Autti-Rämö, I., Fagerlund, Å., et al. (2007). Unique facial features distinguish fetal alcohol syndrome patients and controls in diverse ethnic populations. *Alcoholism: Clinical and Experimental Research, 31*(10), 1707-1713.

Powrozek, T. A., & Zhou, F. C. (2005). Effects of prenatal alcohol exposure on the development of vibrissal somatosensory cortical barrel network. *Brain research. Developmental brain research*, *155*, 135-146.

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Sowell, E. R., Johnson, A., Kan, E., Lu, L. H., Van Horn, J. D., Toga, A. W., et al. (in press). Mapping white matter integrity and neurobehavioral correlates in children with fetal alcohol spectrum disorders. *Journal of Neuroscience*.

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Zhou, F. C., Sari, Y., & Powrozek, T. A. (2005). Fetal alcohol exposure reduces serotonin innervation and compromises development of the forebrain along the serotonergic pathway. *Alcoholism: Clinical and Experimental Research, 29*, 141-149.

Publications under review

Fang, S., McLaughlin, J., Fang, J., Huang, J., Autti-Ramo, I., Fagerlund, A., et al. (2007). *Automated diagnosis of fetal alcohol syndrome using 3D facial image analysis*. Manuscript submitted for publication.

Mattson, S. N., Riley, E. P., Autti-Rämö, I., May, P. A., Konovalova, V., Jones, K. L., et al. (2007). *Spatial learning and navigation deficits in an international sample of children with heavy prenatal alcohol exposure*. Manuscript under revision after review.

McLaughlin, J., Fang, S., Huang, J., Jacobson, S. W., Hoyme, H. E., Robinson, L. K., et al. (2007). *Interactive feature visualization and detection for 3D face classification*. Manuscript submitted for publication.

Zhou, F., Yuan, F., & Goodlett, C. (2007). *Dysraphia and serotonin neuronal reduction induced by prenatal alcohol exposure is reduced by peptidergic agonist of activity dependent neurotrophic factor*. Manuscript submitted for publication.

Posters and Presentations:

Miller, K. W., & Charness, M. E. (2007). *Alcohol and the L1 cell adhesion molecule*. Presented at the Winter Conference on Brain Research, Snowmass, CO.

Miller, K. W., & Charness, M. E. (2007). *Overview: Fetal alcohol spectrum disorders*. Presented at the NIDA Workshop: Intrauterine Exposure to Drugs and Alcohol: What is the Next Step for Medication Research & Treatment?, Bethesda, MD.

Miller, K. W., & Charness, M. E. (2007). *Alcohol and the L1 cell adhesion molecule*. Presented at the Department of Pharmacology Seminar, Medical College of Virginia, Richmond, VA.

Miller, K. W., & Charness, M. E. (2007). *Alcohol and the L1 cell adhesion molecule.* Presented at the Ernest Gallo Clinic and Research Center, UCSF, Emeryville, CA.

Miller, K. W., & Charness, M. E. (2007). *Alcohol and the L1 cell adhesion molecule.* Presented at the Department of Neuroscience and Physiology, State University of New York, Syracuse, Syracuse, NY.

Miller, K. W., & Charness, M. E. (2007). *Alcohol and the L1 cell adhesion molecule.* Presented at the Center for Drug and Alcohol Programs, Medical University of South Carolina, Charleston, SC.

Sulik, K. (2007, October). *High resolution MRI results regarding the CNS effects of acute gestational day 7 versus day 8 ethanol exposure.* Presented at the NIAAA/INSERM meeting regarding U.S./French Research Collaborations in Alcohol Abuse and Alcoholism, Rockville, MD.

Myers, L. (2007, November). *Results of MRI work to date*. Presented at the UNC Toxicology Department Seminar, Chapel Hill, NC.

Sulik, K. (2007, November). *Description of the results of our studies to date.* Presented to UNC Pathology graduate students, Chapel Hill, NC.

Sulik, K. (2007, December). *Description of the results of our studies to date.* Presented to UNC first year medical students, Chapel Hill, NC.

Flury, L., Rogers, J., Foroud, T., Robinson, L., & Moore, E. (2005). Fetal alcohol syndrome and 3-D facial imaging: a preliminary classification study [Abstract]. *Alcoholism: Clinical and Experimental Research, 29*(Suppl. 5), 129A.

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Mattson, S., Flury-Wetherill, L., Foroud, T., Rogers, J., Moore, E., Autti-Rämö, I., et al. (2007). Classification of fetal alcohol syndrome using 3-D facial imaging and neuropsychological data [Abstract]. *Alcoholism: Clinical and Experimental Research, 31*(Suppl. 6), 246A.

Ward, R. E., Moore, E. S., Wetherill, L., Foroud, T., & the CIFASD Consortium. (2007). *Population specific craniofacial variation and its relevance to the clinical application of anthropometry [Abstract]*. Abstract submitted for presentation at the meeting of the American Association of Physical Anthropologists.

Moore, E. S. (2007, March). *The use of three-dimensional facial images to identify FASD across ethnic groups*. Lecture presented at the 2nd International Conference on Fetal Alcohol Spectrum Disorder, Victoria, BC.

Ward, R. E. (2007, April). *Screening children for fetal alcohol spectrum disorders using 3-D laser photography*. Lecture presented at the meeting of the Midwest Association of Toxicology and Therapeutic Drug Monitoring.

Mattson, S. N., Fryer, S. L., Spadoni, A., Bazinet, A., Sowell, E. R., & Riley, E. P. (2008, February). *Functional brain changes in children and adolescents with heavy prenatal alcohol exposure*. To be presented at the meeting of the International Neuropsychological Society, Waikaloa, HI.

O'Hare, E. D., Lu, L. H., Bookheimer, S. Y., McCourt, S. T., Houston, S. M., Mattson, S. N., et al. (2007, November). *Increased dorsal frontal and inferior parietal activation during verbal working memory among children and adolescents with prenatal alcohol exposure*. Poster session presented at the 37th annual meeting of the Society for Neuroscience, San Diego, CA.

Mattson, S. N., Roesch, S. C., Riley, E. P., Adnams, C., Autti-Rämö, I., Fagerlund, Å., et al. (2007). Neurobehavioral profile of children with heavy prenatal alcohol exposure [Abstract]. *Alcoholism: Clinical and Experimental Research, 31*(6), 185A.

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McGee, C. L., Vaurio, L., Riley, E. P., & Mattson, S. N. (2007). Comparison of multiple measures of intelligence in children with heavy prenatal alcohol exposure and non-exposed controls [Abstract]. *Alcoholism: Clinical and Experimental Research, 31*(6), 106A.

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O'Hare, E. D., Lu, L. H., McCourt, S. T., Bookheimer, S. Y., & Sowell, E. R. (2007). Evidence for frontal-cerebellar involvement in verbal working memory in typically developing children and adolescents [Abstract]. *NeuroImage Abstracts*.

Kfir, M., Hull, A., Yevtushok, L., Onishchenko, S., Wertelecki, W., Bakhireva, L., et al. (2007, March). *Evaluation of early markers of prenatal alcohol exposure*. Presented at the American Institute of Ultrasound in Medicine Annual Convention, New York, NY.

Jones, K. L., Hull, A. D., Bakhireva, L. N., Wertelecki, W. W., Yevtushok, L., Sosynyuk, Z., et al. (2007). Fetal ultrasound measures as predictors of alcohol-related physical features in the newborn: Preliminary results [Abstract]. *Birth Defects Research Part A: Clinical and Molecular Teratology, 79*, 373.

Chambers, C. D. (2007). Measuring worldwide incidence of FASD [Abstract]. *Birth Defects Research Part A: Clinical and Molecular Teratology, 79*, 384.

Kfir, M. (2007, November). Platform presentation at the 12th World Congress of the International Society of Ultrasound in Obstetrics and Gynecology, New York, NY.

Chambers, C. D. (2007, December). *Fetal alcohol spectrum disorders in Russia and Ukraine - Diagnosis and intervention/prevention.* Presented at the Grand Rounds, University of California, San Diego and Rady Childrens' Hospital.

Chambers project. 3 abstracts submitted to the Teratology Society - 2008

Kfir M, Yevtushok L, Onishchenko S, Wertelecki W, Bakhireva L, Chambers CD, Jones KL, Hull, AD. (2007). Can Prenatal Ultrasound Detect the Effects of In utero alcohol exposure? Oral presentation at 17th World Congress on Ultrasound in Obstetrics & Gynecology held in Florence, Italy, October 7 – 11 2007. *

* Awarded prize for best oral presentation at meeting.