Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD)

Progress Report
January 2009

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

II. Title of Project: Administrative Core of the CIFASD 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.

8. Disseminate new data arising from the consortium.

IV. Methods:

1. Regular monthly conference phone calls conducted through the use of AccuConference.

2. CIFASD website maintained and updated.

3. Biannual Meetings and Progress Reports.

4. Interaction with and help to facilitate goals of Central Repository/Informatics Core.

5. Annual Project evaluations and priority/goal-setting.

6. Exploratory/Developmental Projects: Two developmental projects are included in the Administrative Core. Solicitations for new developmental projects sent in November 2008 and 30 project proposals ideas are currently being reviewed and ranked by the CIFASD.

V. Accomplishments and Results:

1. Since the last progress report in January of 2008, the primary accomplishment of the Administrative Core has been fulfilling its role as liaison for the CIFASD PIs, the Scientific Director, Science Advisory Board members and the NIAAA staff. This was accomplished by providing leadership in the following formats:

a. Monthly Conference Calls: Separate Basic Science and Clinical Research Component conference calls were held monthly and were moderated by the Consortium Coordinator. PIs reported on their monthly progress on each of these calls. Verbatim mp3 recordings of these calls were posted in the secure area of the CIFASD website.
b. Special Conference Calls: The Administrative Core assisted in facilitating smaller special area group conference calls as needed.
c. Biannual Meetings: The Administrative Core arranged the June 2008 and the January 2009 CIFASD meetings. Coordination of these meetings included coordinating participant calendars in date selection, contracting sleeping room rates at selected hotels, reserving meeting space, completing travel arrangements for the Consortium Coordinator, Scientific Director, Science Advisory Board members and invited guests, collating and distributing meeting materials and researching and inviting outside experts to the meetings.

d. Progress Reports solicited and collated for each winter biannual meeting. Progress Reports and PPT presentations are posted in the secure area of the CIFASD website.
e. Various communications with PIs, the Scientific Director, Science Advisory Board members and the NIAAA staff as needed in the forms of phone calls, emails and face-to-face meetings.

2. Another critical accomplishment of the Administrative Core is its role of updating and maintaining the CIFASD website. Latest news and upcoming events sections are updated on a monthly basis. The publication section was updated biannually once progress reports were received.

3. The Administrative Core facilitated the recruitment of new scientists and introductions to new technologies to the consortium through guest presentation invitations at the biannual meetings. These invitations were also extended to experts in fields of interest to members of the consortium.

    a. At the June meeting, Alexandre Medina and William Guido from Virginia Commonwealth University presented their research findings to the group. They have subsequently submitted a developmental project proposal to the CIFASD which are currently under review.

b. At the June meeting, Cynthia Bearer from Case Western Reserve spoke on recent developments of biomarkers and Barbara Finlay from Cornell University spoke on comparative brain development, both experts in their respective fields.
c. The June meeting had a full day devoted to a joint PASS (Prenatal Alcohol, SIDS, and Stillbirth)/CIFASD Steering Committee Joint Session in which members of the CIFASD and PASS were able to present their findings to each other and discuss areas of research similar to both groups which also provided an opportunity for discussion of future research collaborations.
d. At the upcoming January meeting, Peter Hammond from the Institute of Child Health UCL – London, UK will be presenting on computational models of facial dysmorphology and Christian P. Klingenberg from the University of Manchester, UK will present on the study of size and shape variation of morphometric features. Hannah Kinney from Children's Hospital Boston will be providing an update on the PASS projects.

4. The Administrative Core PI has given several talks about the consortium educating the community and providing scientific outreach on behalf of the CIFASD. In addition, the Consortium Coordinator organized a CIFASD Plenary Session for the 3rd International Conference on Fetal Alcohol Spectrum Disorder Integrating Research, Policy, and Promising Practice Around the World: A Catalyst for Change – Victoria, BC, Canada March 11th – 14th, 2009.

THURSDAY, MARCH 12

"Confidence, like art, never comes from having all the answers; it comes from being open to all the questions" *Earl Gray Stevens*

8:00am Registration & Exhibits Open

PLENARY SESSION

8:30am Opening Remarks – Hans-Ludwig Spohr

Hans-Ludwig Spohr, Head of Department (e.m.), Department of Pediatrics, DRK-Kliniken Westend; apl. Professor, Humboldt University Berlin, Berlin, Germany

8:45am Research – Collaborative Initiatives on FASD

Moderator: Sterling K. Clarren MD, FAAP; CEO and Scientific Director, Canada NW FASD Research Network; Clinical Professor of Paediatrics, University of British Columbia; Clinical Professor of Pediatrics, University of Washington, Seattle, WA

An International Approach to Research
Edward Riley PhD; Director, Center for Behavioral Teratology, San Diego State University; and, Distinguished Professor, Department of Psychology, San Diego State University, San Diego, CA

Prenatal Diagnosis of FASD: Is it Possible?
Andrew D. Hull MD, FRCOG, FACOG, Associate Professor of Clinical Reproductive Medicine, Department of Reproductive Medicine, University of California, San Diego, San Diego, CA

Application of Magnetic Resonance Microscopy to an FASD Model
Kathleen K. Sulik PhD; Professor, Cell & Developmental Biology and Bowles Center for Alcohol Studies, The University of North Carolina at Chapel Hill, Chapel Hill, NC

A Multisite Neurobehavioural Assessment of FASD
Sarah Mattson PhD; Associate Director, Center for Behavioral Teratology, San Diego State University; and, Professor, Department of Psychology, San Diego State University, San Diego, CA

Can We Improve Our Ability To Detect Fetal Alcohol Syndrome? Using 3D Facial Images
Tatiana Foroud PhD, P. Michael Conneally Professor of Medical and Molecular Genetics, Indiana University School of Medicine; and Director of Hereditary Genomics Division, Indiana University School of Medicine, Indianapolis, IN

5. Solicitations were sent for CIFASD Developmental/Exploratory projects to FASD Study Group and RSA listservs. Thirty 1-page project proposals were received. CIFASD members from both the Basic Science and Clinical Components volunteered to evaluate the proposals on their scientific merit and their ability to relate to the mission of the CIFASD. Coordination of 1-page project proposals into a PDF and an Excel sheet ranking system created and distributed to all volunteers from the PI.

6. The Administrative Core has provided assistance and leadership to various projects and CIFASD committees as needed. Financial assistance was provided to Dr. Cudd's project to support additional fetal ultrasounds on sheep. Facilitation and coordination of a visit to Dr. Cudd's College Station laboratory by Dr. Hull was planned for mid-January 2009. Funds for the continuation of Dr. Miller's project were allocated to Dr. Charness with hopes of this project being submitted as a U01 in 2009.

7. Two smaller CIFASD groups were created as a result of discussions from the June 2008 biannual meeting, the Tissue Banking Committee and the Alcohol Use

Questionnaire Committee. The Administrative Core PI provided guidance to these committees as needed and kept them on track to complete their goals.

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 developmental/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 again 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. Another main responsibility of the Administrative Core this year will be to facilitate and oversee the selection of new developmental projects.

IX. Publications:

Not applicable.

X. Posters and Presentations:

"FASD: Not Just Another Pretty Face: Effects of Prenatal Alcohol on Brain & Behavior," presented at the Department of Psychiatry Grand Rounds at the University of Florida College of Medicine – Gainesville, October, 2008.

"Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD)," presented at INSERM/NIAAA Symposium, Paris, France, October, 2008.

"Etiology and occurrence of FAS/FASD," presented at AFA FAS/FASD Workshop, Uppsala, Sweden, September, 2008.

"Prenatal Alcohol Exposure and Brain Development," presented at the FASD Physician Training and Conference, Oxnard, CA, September, 2008.

"The Foetal Brain & Alcohol – Defining Foetal Alcohol Spectrum Disorder (FASD)," presented at the Parents for Children Consequences for Children Affected by Maternal Drug & Alcohol Usage: A Multi-Disciplinary Approach conference, London, UK, September, 2008.

"Recent Trends in FASD Research: What are we Learning," presented at The ARC of Illinois Fetal Alcohol Spectrum Disorder Conference: Prevention, Information & Education, Chicago ARC conference, Alsip, IL, August, 2008.

"Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD)," presented at the U.S./Asian Collaborations in Alcohol Research meeting, Washington, DC, July, 2008.

"FASD: Not Just Another Pretty Face: Effects of Prenatal Alcohol on Brain & Behavior," presented at the Northern California Regional Chapter of the Society of Toxicology meeting, San Francisco, CA, April 2008.

"FASD: The Effect of Prenatal Alcohol Exposure on Brain and Behavior," presented at Midwest Regional Fetal Alcohol Syndrome Training Center Booster Session, St. Louis, MO, April, 2008.

"FASD: Not Just Another Pretty Face: Effects of Prenatal Alcohol on Brain & Behavior," presented at Parents for Children Consequences for Children Affected by Maternal Drug & Alcohol Usage: A Multi-Disciplinary Approach conference, London, UK, March, 2008.

"New Intervention: Bimanual Coordination & Exercise," presented at Parents for Children Consequences for Children Affected by Maternal Drug & Alcohol Usage: A Multi-Disciplinary Approach conference, London, UK, March, 2008.

"Prenatal Alcohol Exposure: Imaging the Damage, Mitigating the Effects – Is There Hope Beyond Prevention?" presented in Bakersfield, CA, January, 2008.

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

II. Title of Project: Informatics Core for the Collaborative Initiative on Fetal Alcohol Spectrum Disorders U24 AA014818

III. Objectives:

The objective of the Informatics Core is to support the aims of the Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD) by enabling the collection, sharing, and analysis of data. In particular, we aim to facilitate the creation of data dictionaries that ensure that data of different types and from different locations can be shared and integrated and we aim to provide a reliable and HIPAA-compliant central repository and other tools to facilitate data entry, data storage, and data retrieval.

IV. Methods:

CIFASD is collecting several different types of data: Dysmorphology, Neurobehavior, 2D facial images, 3D facial images, Demographics, 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 to define a data dictionary that precisely defines what data are to be collected.

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, 2008.

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	Data Dictionary	**Updated ***
	MS Access Input Tool	**Updated ***
	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* *
Demographics	Data Dictionary	*In progress* *
	MS Access Input Tool	*In progress* *
	Central Repository Schema	*In progress* *
	Upload Tool	*In progress* *
	Report Tool	*In progress* *
	Data Dictionary	*In progress* *
3D Facial Images	Data Dictionary	**Finished**
	Central Repository Schema	**Finished**
	Upload Tool	**Finished**
	Report Tool	**Finished**
2D Facial Images	Data Dictionary	
	Central Repository Schema	
	Upload Tool	
	Report Tool	
Ultrasound	Data Dictionary	**Finished**
	MS Access Input Tool	**Finished**
	Central Repository Schema	
	Upload Tool	
	Report Tool	
Screener	Data Dictionary	**Finished**
	MS Access Input Tool	**Finished**
Follow-up/Outcome	Data Dictionary	**Finished**
	MS Access Input Tool	**Finished**
Infant Neurobehavior [Bayley & Maternal	Data Dictionary	**Finished**
	MS Access Input Tool	**Finished**

Questionnaire]	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 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.

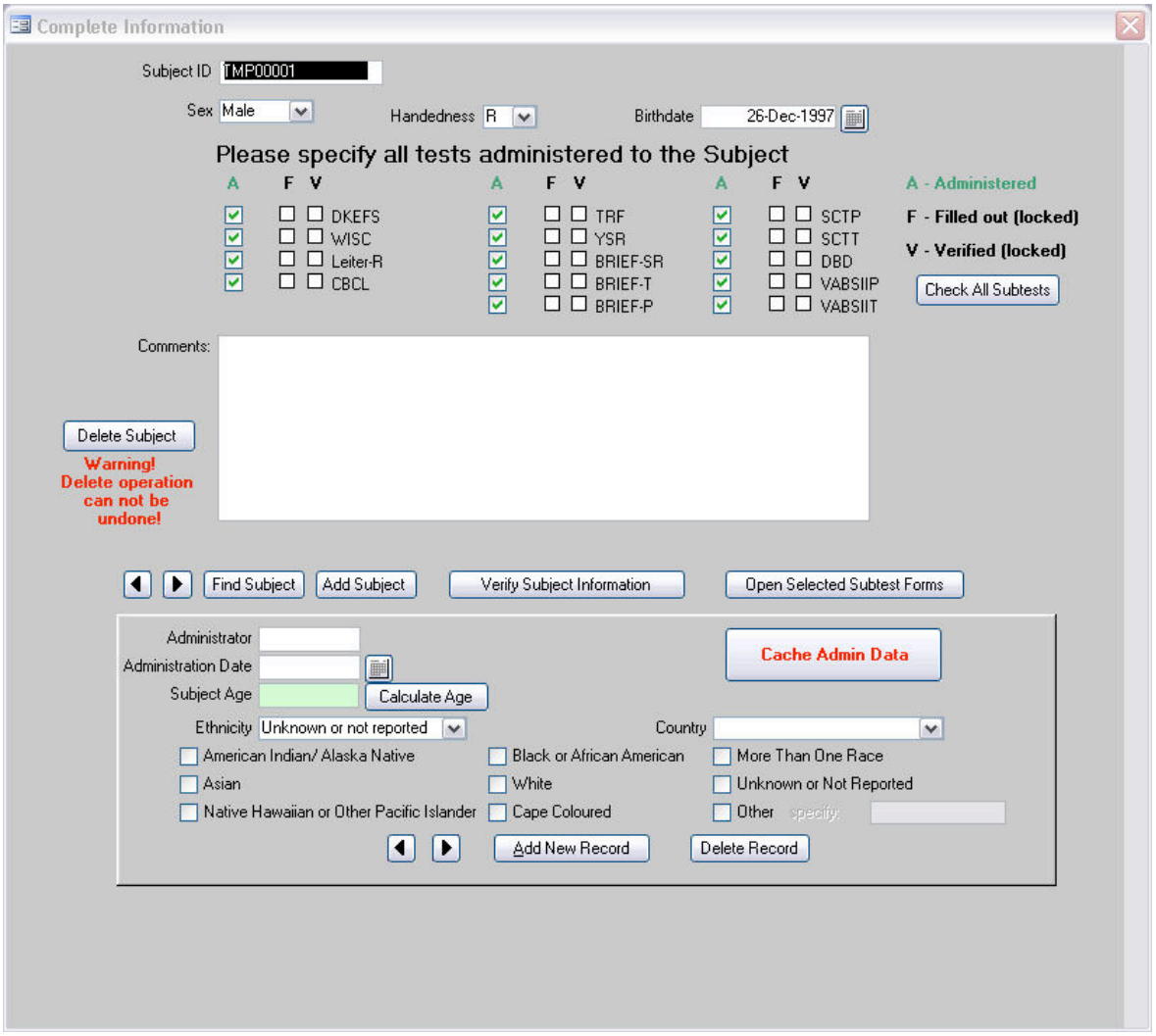


Figure 1: Neurobehavior Phase II Data Entry Input Tool. This data entry form includes double-entry data validation functionality to decrease data entry errors as well as range checking that makes it impossible to enter values outside the permissible ranges identified in the data dictionary.

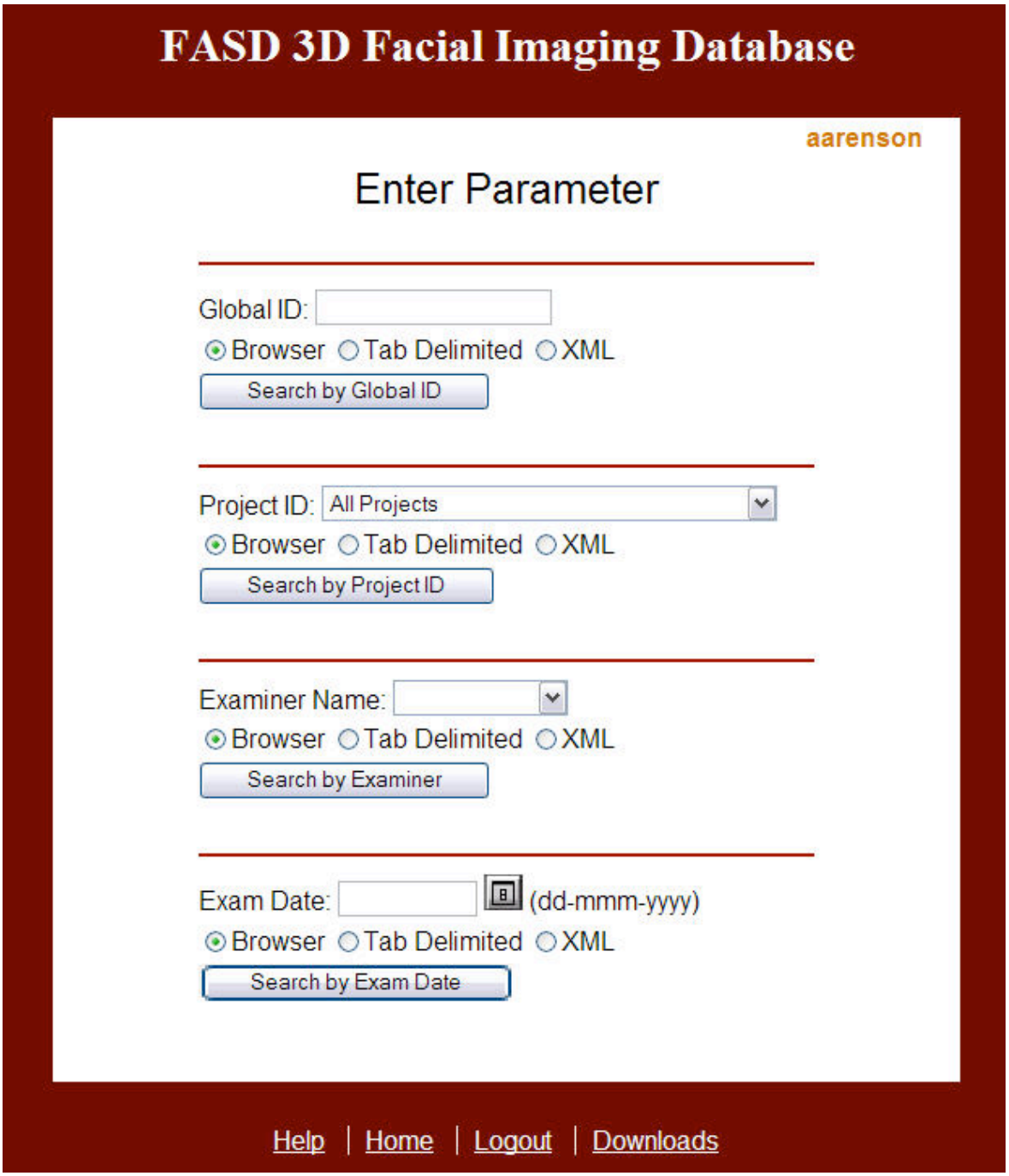


Figure 2: Web-based 3D facial imaging data access and retrieval tool.

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:

With the inception of the grant in September, 2007, there was an immediate need for rapidly creating new software tools. After working with the Administrative Core and the various projects in the previous period to navigate the conflicting priorities across the consortium as to which software tools were needed and when, the Informatics Core had produced the Bayley and Maternal Questionnaire variables sections of the Infant Neurobehavior Input Tool and begun creation of the Neurobehavior phase II Input Tool.

The Informatics Core began this period with the ambitious goal of completing the 700+ variable Neurobehavior phase II Input Tool by mid March and successfully reached this goal. The Informatics Core has also subsequently expanded the CIFASD Central Repository to accommodate Neurobehavior phase II data, including providing submission and reporting tools. User acceptance testing for the Neurobehavior phase II Input, Upload, and Query tools are close to finishing.

The next major piece of software development was the creation of the Demographics data dictionary and the Demographics Input, Upload, and Query tools. Given the pressing need to provide these software tools to the collaboration combined with a limited amount of time available from the collaboration members who were responsible for providing guidance on the requirements for the Demographics data, the Informatics Core took the unusual step of providing a prototype of the Demographics Input Tool before completing the Demographics data dictionary. This action balanced the risk of potentially wasted effort if the prototype proved to be unusable versus the likely delay to the collaboration of not beginning work on the Demographics Input Tool. The result of the action was highly positive with most of the prototype proving usable. The Demographics tools are in the final stages of testing and refinement.

Another major emphasis in this period was investigating methods to improve the assurance of Dysmorphology diagnoses. The Dysmorphology data was found to suffer from a variety of concerns, including too little data having been recorded, such that the diagnosis recorded by an expert Dysmorphologist was not always supported by the other data recorded by that Dysmorphologist. The diagnosis of FAS made by the expert Dysmorphologists depends on a complicated set of conditions based on may different variables. The Informatics Core prepared the hardware and software required to conduct a pilot study to help improve data collection. The pilot study involved having a Dysmorphologist enter data during an examination directly into a laptop computer rather than recording data on a paper form. The Informatics Core updated the Dysmorphology Input Tool such that it would provide immediate feedback on whether or not the data that were recorded supported the diagnosis that was recorded. The Informatics Core also secured the laptop computer both for patient privacy issues and for disaster recovery issues. The pilot study found the use of the computer interface to be too slow to keep up with the pace of examinations, but to otherwise be successful. Whether or not to revisit this issue has not yet been decided by the consortium.

To support the collaboration's need to combine different types of data, the Informatics Core created the Cross Query -- new functionality for retrieving data from the CIFASD Central Repository. Working in conjunction with a committee of representatives from multiple projects, the Informatics Core designed a system that allows a wide degree of flexibility in choosing the

subset of data to be retrieved. The Cross Query is currently in beta testing, with representatives from the committee providing feedback on ways to extend the existing functionality.

Other activities in this period include updates to the Alcohol and Control and the Followup/Outcome input tools to alter the way in which missing data are handled and what affects missing data have on calculated fields. This work is in early stages, having only recently begun.

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

The Informatics Core is essential infrastructure for CIFASD as a whole. The structure of a separate Informatics Core has facilitated the collaborative processes that have enabled the consortium's scientists to come to consensus on data definition and measurement issues that are essential to the broader goals of the CIFASD as a whole. We believe that 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 6 Months:

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.

Priorities for the next six month include finalizing the Input, Upload, and Query tools for the Neurobehavior phase II data and for the Demographics data, expanding the Infant Neurobehavior Input tool to accommodate heart rate monitoring variables, updating the Alcohol and Control and Followup/Outcome Input tools to better handle missing variables, and expanding the Ultrasound Input tool to include biophysical profiling variables.

IX. Publications:

n/a

X. Posters and Presentations:

n/a

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

II. Title of Project: Dysmorphology Core U24 AA014815

III. Objectives:

The objective of the Dysmorphology Core is to support all sites contributing to the Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD) by implementing a standard comprehensive protocol for physical examination of all children. In addition, we propose to address hypotheses regarding the relationship between FASD-related structural features identified through standard physical examinations and neurobehavioral development as well as complimentary diagnostic methods that are being tested in other Consortium sites.

The specific aims are as follows:

1. To assure consistency as well as accuracy in recognition of FASD at all CIFASD project sites where children are being evaluated.

2. To delineated the full range of structural anomalies in children prenatally exposed to alcohol in order to determine the boundaries that encompass FASD in prospective as well as retrospective studies in the Consortium.

3. To identify specific structural defects or clusters of features that are predictive of or correlated with deficits in neurobehavioral development across development 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 brain development.

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:

Specific Aims I and II: Over the last year we have provided accurate recognition of FASD at sites in Ukraine, the Great Plains, and San Diego, CA and have scheduled evaluation of children in Atlanta, Georgia on December 4 and 5, 2008 and in Los Angeles. CA on January 5 and 6, 2009. During trips to Ukraine, we have expended a considerable amount of time interacting with pediatricians and geneticists in the Rivne Oblast. We have been very pleased to note that our examination of children has been completely consistent with their examination performed at the time of birth. We have also been involved in re-training pediatricians in the Rivne Oblast.

Specific Aims II and III: As more individual children in the Consortium receive both neurobehavioral assessment and evaluation by the Dysmorphology Core, it is becoming more possible to correlate features identified on the physical examination with neurobehavioral deficits and to determine which specific physical features are predictive of neurobehavioral deficits. These studies are being done in conjunction with the Multisite Neurobehaviorl Assessment of FASD. At present, sufficient standardized physical examinations and neurobehavioral assessments have not been completed to allow sufficient analysis to make conclusions.

Specific Aim 4: We have completed a study correlating maternal drinking during pregnancy with prenatal ultrasound findings . This study has been accepted for publication. In addition, we are involved in a study examining the ability to recognize FAS from 3D facial imaging . For this study two dysmorphologists evaluated 3D facial images of children who they had previously examined in order to determine if they could diagnosis FAS using 3D facial imaging. The results of this study are being analyzed.

VI. Discussion:

The Dysmorphology Core has helped to facilitate the goals of the Consortium and some of the individual projects primarily through traveling to Consortium sites and performing standardized

physical examinations on children ascertained at the individual sites. At San Diego State University 20 children have been evaluated, in Atlanta Georgia 14 children will be evaluated on December 4 and 5, 2008, 8 children have been evaluated in Rivne, Ukraine and 3 children have been evaluated in the Great Plains states.

In addition, a number of talks and training sessions have been done in Ukraine.

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

The Dysmorphology Core has interrelated with a number of other projects in the Consortium including the 3D Facial Imaging Project, the Spectrum of and Nutritional Risk Factors for FASD in Russia and Ukraine Project, and the Multisite Neurobehavioral Assessment of FASD Project.

VIII. Plans for Next Year:

We will continue to see children ascertained at all CIFASD sites throughout the world and have a time set for evaluation of children in Los Angeles on Jan. 15 and 16, 2009. We are planning to complete the analysis of data regarding the relationship between FASD-related structural features identified through standard physical examination and 3D facial imaging . We also will began correlating the relationship between FASD-related structural defects identifies through the standard physical examination and neuro-imaging studies. Finally it is important that we begin correlating the FASD-related structural features with the neuro-behavioral findings.

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 andGynecology.

X. Posters and Presentations:

None

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, known to influence CNS development. Accumulating evidence indicates that perinatal choline deficiency impairs brain development whereas perinatal choline supplementation can alter CNS structural, neurochemical and electrophysiological development to produce long-lasting improvements in cognitive functioning. Previous studies have shown that perinatal choline supplementation can reduce the severity of some fetal alcohol effects. However, it is not known if choline supplementation restricted to late gestation is effective, whether ethanol induces choline deficiency and whether deficiencies in dietary choline exacerbate ethanol's teratogenic effects. 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 micronutrient intervention study, which includes a choline supplementation component. To determine whether choline supplementation initiated during late gestation mitigates ethanol's teratogenic effects, pregnant Sprague-Dawley rats are exposed to 6.0 g/kg/day ethanol from gestational days (GD) 5-20 and pups are exposed to 5.25 g/kg/day from postnatal days (PD) 2-9, to model alcohol exposure throughout gestation (all three trimesters). Pair-fed and ad lib lab chow controls are included. Following birth, ethanol-exposed subjects are treated with various doses of choline (15-150 mg/kg/day) from PD 1-21. This study will help us identify the dose-response effects of choline supplementation administered during the late gestational/early postnatal period.

2. Specific Aim 2: To determine whether choline deficiency exacerbates ethanol's teratogenic effects, dams are fed a diet containing 40, 70, or 100% recommended levels of choline, levels that are consistent with those reported in epidemiological studies and are, thus, clinically relevant. 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. Physical, behavioral, and hippocampal development is examined in the offspring. Secondly, it is possible that alcohol itself reduces choline metabolism and utilization. Our lab is collecting brain, liver and blood from pregnant dams (ethanol-exposed, pair-fed and lab chow controls) and fetuses, sending the tissue to the laboratory of Dr. Carl Keen for analyses of choline and associated metabolites.

V. Accomplishments and Results:
This project has been funded for 1 year and 3 months.

1. Specific Aim 1: To date, we have generated 54 litters with 4-9 subjects/sex per group. The final subjects will be generated within the next 2 months and this initial study will be completed by the spring. The initial data suggest that choline supplementation, even at levels of 15 mg/kg/day, can reduce the severity of some, but not all fetal alcohol effects. Specifically, choline supplementation from PD 1-21 does not appear to reduce the severity of overactivity in the open field activity, but did reduce the severity of spatial learning deficits on the Morris water maze (see Figure 1).

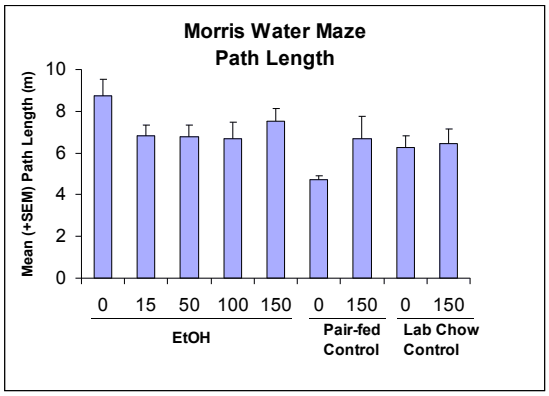


Figure 1. Ethanol-exposed subjects given no choline (0 mg/kg/day) tend to be take longer to find the escape platform compared to all other groups, including ethanol-exposed subjects treated with choline (15-150 mg/kg/day).

2. Specific Aim 2: We have completed the choline deficiency study and are currently working on final data analyses and manuscript preparation. In general, the combination of prenatal alcohol exposure and 40% choline diet produced the most severe alterations in physical and behavioral development (see Figures 2-4). We also 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.

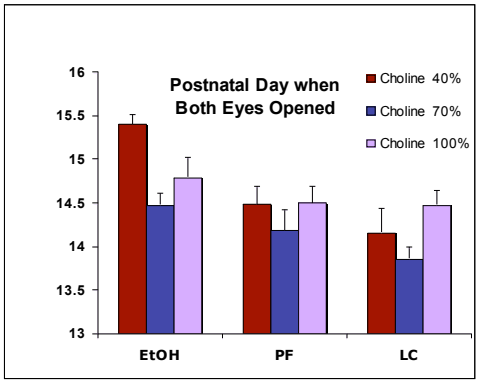


Figure 2. The combination of choline deficiency (40% recommended levels) and prenatal alcohol delayed eye opening.

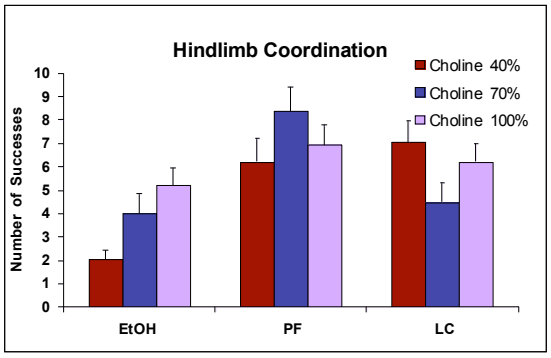


Figure 3. The combination of choline deficiency and prenatal alcohol exposure significantly impaired hindlimb coordination.

Sample data from a control subject are shown in Figure 5. Dr. Keen's group has recently completed an assay for phosphatidylcholine levels, so we will now beginning to generate additional subjects.

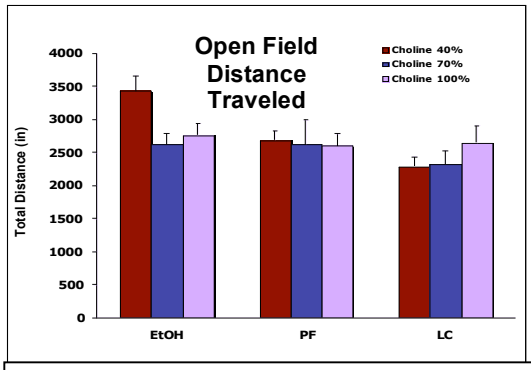


Figure 4. The combination of choline deficiency and prenatal alcohol exposure produce significant increases in open field activity levels.

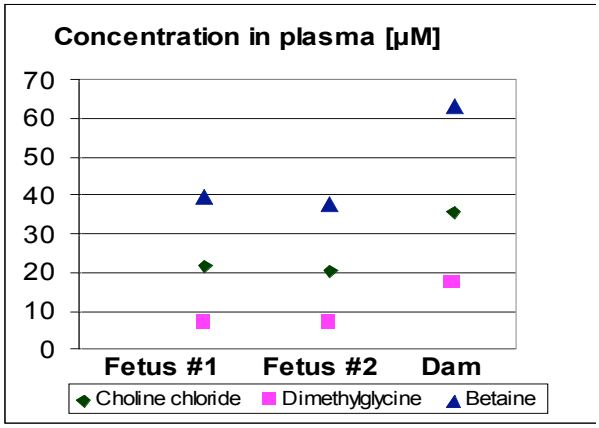


Figure 5. Plasma levels of choline chloride and metabolites from a control dam and fetuses.

VI. Discussion:

Findings from Specific Aim #1 suggest that the dose levels of choline supplementation being used in Chambers' clinical study are adequate for reducing some fetal alcohol effects. Final analyses will indicate the range of behavioral domains that may be improved. Understanding the parameters that control choline's effectiveness is critical for developing larger scale intervention studies and hopefully will lead to a successful treatment for individuals exposed to alcohol prenatally. Findings from Specific Aim #2 illustrate that nutritional factors influence alcohol's teratogenic effects. The dietary choline levels used in our study represent suboptimal choline that is well within the levels observed in epidemiological studies both in the U.S. and abroad and do not represent severe choline deficiencies. These data suggest that the combination of suboptimal levels of a single nutrient can exacerbate ethanol's teratogenic effects. Elucidation of how ethanol interacts with nutritional factors, will improve identification of high risk populations as well as the development of more effective intervention strategies both during and subsequent to prenatal alcohol exposure.

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 prenatal alcohol and/or 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:

We will complete the choline supplementation study and follow-up with a study restricting choline supplementation to PD 1-9. We will also generate subjects for tissue collection to measure the effects of alcohol and diet on choline and choline metabolite levels in brain, blood and liver.

IX. Publications:

None.

X. Posters and Presentations:

Nacah, Y., Arce, M., & Thomas, J.D. (2009). Dietary choline deficiency exacerbates the effects of prenatal alcohol exposure on physical and behavioral development. Presentation at the annual meeting of the Western Psychological Association.

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.

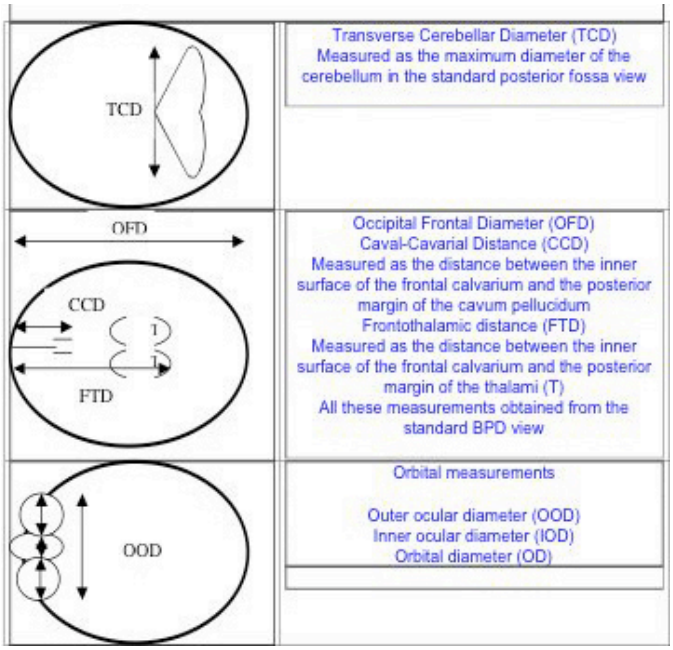
1. To correlate BPP and startle responses with postnatal assessment of growth and structural features as assessed by a standardized dysmorphological examination
2. To correlate BPP and startle responses with altered neurobehavioral assessment at 6 & 12 months
3. To assess the dose response relationship 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 were taught how to obtain the BPP and use the standard scoring system. Spontaneous and evoked startle responses are being recorded .Live born 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.

Figure 1 Specific CNS Measurements



V. Accomplishments and Results:

1. A week long trip was made to Ukraine by the PI in March 2008 and a further visit by the principal collaborators in November 2008.

2. The additional ultrasound measures of BPP and startle responses have been added to the protocol for all newly recruited subjects

3. Analysis of the prenatal ultrasound pilot data with respect to alcohol exposure have been completed and are due to be published in ***Ultrasound in Obstetrics and Gynecology*** in 2009**.**

4. Analyses of prenatal ultrasound measures and physical features of FASD have also been completed and a manuscript is in preparation.

5. Groundwork for further expansion of imaging studies in Ukraine has been laid. The Rivne site had obtained a new state of the art 3DUS machine at the time of our visit and the possibility of using the machine for 3DUS studies was explored. Several training sessions occurred using the new machine and sample 3D volume data was collected for evaluation off site. These data sets were successfully evaluated on our return to the USA supporting the potential for further expansion of imaging in Ukraine with onsite data collection and transmission of data to San Diego for detailed manipulation and analysis. In November 2008 two further 3DUS machines were purchased by the Oblast making a total of three.

6. Recruitment into the study is progressing very well with about 90 additional subjects enrolled so far. The site is continuing to enroll about 20 subjects per month and we are well on target to reach our goal of 360 new subjects by December 2009.

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 have begun the BPP and startle assessments in tandem with morphometric and morphologic measurements.

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

The PI continues to work 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.

Following a successful joint meeting with the PASS study investigators the PI has developed a protocol for evaluation of the fetal EKG in utero in subjects at risk of FASD. This has been submitted to the Consortium as a developmental project and provides collaborative opportunities within the Consortium and with Dr William Fifer a PI in the PASS study.

A joint abstract with Dr Kathy Sulik was submitted and accepted for oral presentation at the Annual David W. Smith Workshop on Malformations and Morphogenesis. (see below). Further collaborations are planned.

VIII. Plans for the Next Year:

The PI will visit Ukraine in February 2009 together with his research sonographer from UCSD. Progress assessment and project supervision is planned. together with opportunities for problem solving. The Rivne site has obtained a total of three new ultrasound machines, all state of the art with three dimensional imaging capabilities. The PI has submitted a developmental project to the Consortium to explore the use of 3DUS in FASD.

As more subjects deliver and their newborns are available for evaluation we will move forward with integration of prenatal and postnatal findings.

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).

Sulik KK, O'Leary Moore S, Parnell SE, Myers EA, Dehart DB, Johnson GA, Chambers CD, Hull AD High resolution magnetic resonance imaging of alcohol-exposed fetal mice confirms and informs human prenatal ultrasound studies. (2008) Proceedings of the 29th Annual David W. Smith Workshop on Malformations and Morphogenesis.

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.

Sulik KK, O'Leary Moore S, Parnell SE, Myers EA, Dehart DB, Johnson GA, Chambers CD, Hull AD High resolution magnetic resonance imaging of alcohol-exposed fetal mice confirms and informs human prenatal ultrasound studies. (2008) Oral presentation at the 29th Annual David W. Smith Workshop on Malformations and Morphogenesis.

I. Principal Investigator: Michael E. Charness, M.D.

II. Title of Project: Mutational Analysis of Alcohol Binding Sites on L1; Administrative supplement: U24

III. Objectives: To understand the structural basis for ethanol inhibition of L1 adhesion and the structural requirements for the design of an ethanol antagonist.

IV. Methods:

Mutational Analysis of an Alcohol Binding Site on L1
During the final period of funding of our U01, we used photolabels to demonstrate that there are alcohol binding sites on L1 (Arevalo *et al.,* PNAS 2008). Homology modeling suggested that alcohol might bind at the domain interface between Ig1 and Ig4 to disrupt a hydrogen bond between Y418 on Ig4 and E33 on Ig1, which reside within 2.6 Å of each other. We postulated that this hydrogen bond stabilizes a horseshoe conformation of L1 that favors homophilic binding, and that disruption of the hydrogen bond accounts for ethanol inhibition of L1 adhesion. To test this hypothesis, we generated a double mutant L1 molecule in which Y418 and E33 were each mutated to cysteines (Y418C and E33C). If the interaction between Y418 and E33 is critical for homophilic binding, then we would predict three properties of this double mutant, which we named EY: 1. Under non-reducing conditions, EY will form a disulfide bond between C418 and C33 (C418-S-S-C33), stabilizing the horseshoe conformation of L1 and increasing L1 adhesion; 2. Under reducing conditions, the disulfide bond would break, leaving neither a hydrogen bond nor a disulfide bond to stabilize the horseshoe conformation, thereby reducing L1 adhesion to the same extent as ethanol; 3. The replacement of a hydrogen bond with a disulfide bond between C418 and C33 in EY should make EY less sensitive to ethanol.

V. Accomplishments and Results:

1. Development of a Transient Transfection Assay to Test the Effects of L1 Mutations on L1 Adhesion
We showed previously that clonal cell lines derived from stable transfection of NIH/3T3 cells with human L1 were variably sensitive to ethanol inhibition of L1 adhesion (Wilkemeyer *et al.*, J Neurochem 98). To test the effects of L1 mutations on ethanol inhibition of L1 adhesion, we required an expression system in which ethanol reliably inhibits wild type (WT) L1 adhesion. After considerable experimentation, this proved feasible using transient transfection of NIH/3T3 cells with polyfect, a new transfection reagent from Qiagen. Using an L1-GFP construct as a marker, we demonstrated a transient transfection efficiency of approximately 70%. Western blot analysis demonstrated that after 48 hours of transfection, the levels of L1 expression were similar in WT-L1 and EY cells.

2. Cell Adhesion in WT-L1 and EY Cells under Non-reducing Conditions
Transfected cells were triturated to form a single cell suspension and then agitated on a rotary shaker for 30 minutes to promote cell aggregation. The number of single and adherent cells was determined by counting randomly selected fields under phase contrast microscopy. A majority of the L1-transfected NIH/3T3 cells formed aggregates, whereas the majority of non-transfected NIH/3T3 cells did not. L1-mediated cell adhesion was defined as the difference between the percentage of adherent cells in the L1-transfected NIH/3T3 cells and in the NIH/3T3 cells treated with the transfection reagent alone. L1 adhesion was 55%±14% ($p<0.01$) higher in EY cells than in WT-L1 cells. These findings suggest that the stabilization of the interaction between Ig1 and Ig4 with a disulfide bond favors L1 homophilic binding.

3. Cell Adhesion in WT-L1 and EY Cells under Reducing Conditions
The contribution of the hydrogen bond between E33 and Y418 to L1 adhesion can be assessed by using a reducing agent to break the disulfide bond between C33 and C418 in EY cells. As expected, treatment of WT-L1 cells with 30 mM β-mercaptoethanol (BME), a reducing agent, had no significant effect on L1 adhesion, because there is no disulfide bond at the Ig1-Ig4 interface. These data also suggest that disulfide bonds at other locations in L1 do not contribute significantly to L1 adhesion. In contrast, BME reduced L1 adhesion in EY cells by 58%±11% ($p=0.001$), an effect comparable to that of 100 mM ethanol in WT-L1 cells (below). These data indicate that the hydrogen bond formed between the ethanol binding residues at E33 and Y418 contributes significantly to L1 adhesion, most likely by stabilizing the horseshoe conformation of L1.

4. Effect of Ethanol on L1 Adhesion in L1-WT and EY Cells
A high concentration of ethanol (100 mM) was used to test its maximal effect on L1 adhesion. In more than 20 consecutive experiments, ethanol inhibited L1 adhesion in WT-L1 cells. The average magnitude of inhibition, 64%±5.7%, was comparable to that observed after treatment of EY cells with BME. A dose response curve revealed that ethanol inhibited L1 adhesion with a half-maximal concentration between 5 and 10 mM, the same potency that we reported in stably-transfected NIH/3T3 cells (Ramanathan *et al.*, J Cell Biol, 1996). Ethanol also inhibited cell adhesion in EY cells, but the maximal effect (49%±5.7%; $n=16$; $p=0.0003$) was significantly less than in WT-L1 cells, and the potency was reduced by approximately 3-fold.

VI. Discussion:

These data indicate that replacement of the hydrogen bond between E33 and Y418 with a disulfide bond reduces the efficacy and potency of ethanol in inhibiting L1 adhesion and suggest that these ethanol binding residues are functionally significant in mediating the effects of ethanol on L1 adhesion.

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

A major goal of the consortium is the development of interventions that will prevent or mitigate the effects of ethanol on fetal development. Our experiments will help provide a better understanding of the structural requirements for the development of ethanol antagonists.

VIII. Plans for the Coming Year

In the coming year of funding, we will continue to analyze the role of individual amino acid residues in the alcohol binding pocket at the domain interface between Ig1 and Ig4 of L1. Our plan is to submit an RO1 to support this work within one year.

IX. Publications:
X. Posters and Presentations:

An abstract for RSA will be submitted.

I. Principal Investigator: Timothy A. Cudd, Ph. D.

II. Title: Translational Studies of FASD Using a Sheep Model U01 AA017120

III. Objectives:

The goals of the CIFASD consortium are to identify sources of variable phenotypes (facial dysmorphology, structural brain damage and neurobehavioral functional deficits) resulting from prenatal alcohol exposure to improve diagnosis and early identification of FASD, and to develop early interventions that may limit adverse outcomes in at-risk pregnancies. Experimental animal models are essential to those goals. Rodent studies have identified causal links between alcohol exposure factors (dose, pattern, developmental timing) and the type and extent of teratogenic phenotypes (facial dysmorphology, brain damage, and neurobehavioral deficits). Rodent models are limited, though, in that they cannot provide an *in utero* model of alcohol exposure that extends into the human 3rd trimester, because brain development comparable to that of the human 3rd trimester occurs entirely after birth in rats and mice. These species differences limit the suitability of rodents for translational studies investigating effects of drinking during the 3rd trimester. This is a critical limitation, because binge drinking that continues into the 3rd trimester is associated with increased risk or severity of adverse outcomes on brain and behavior, and may be a key source of neurobehavioral variability in FASD. Likewise, treatments intended to reduce the *in utero* effects of prenatal alcohol exposure in humans likely will extend into 3rd trimester, and thus are best evaluated in a species in which the stages of prenatal brain development comparable to all three trimesters in humans also occur *in utero*. The sheep is one such species that offers several key advantages for experimental studies addressing the goals of the consortium.

This project involves the novel use of a sheep model to fill a critical scientific and translational niche for CIFASD—to bridge basic and clinical studies more closely than has been achieved in the past. There are two long-term objectives. The first is to use the sheep model to compare the phenotypic effects of binge-like alcohol exposure during the period of brain development comparable to that of the human first trimester (*1st-trimester model*), with similar binge-like exposure that extends over the stages of brain development encompassing all three human trimesters (*3-trimester model*). We will compare the two models in terms of effects on growth, facial dysmorphology and brain and behavioral development—the phenotypes used to diagnose fetal alcohol syndrome—to test the hypothesis that more pervasive effects on brain and neurobehavioral development will result from binge exposure that continues after the first trimester. These will use measures and methods derived explicitly from, and collaboratively linked directly to, approaches that have been developed or applied in the human components of the Consortium (e.g., facial morphometrics; structural magnetic resonance brain imaging (MRI); eyeblink conditioning and spatial learning to test neurobehavioral function). The second goal is to test the hypothesis that choline supplementation initiated periconceptually will attenuate or prevent the adverse effects of alcohol exposure in the 3-trimester model. This goal will inform and complement the two other choline-supplementation projects in the consortium, a basic science project using rats (Thomas) and a prospective human clinical study (Chambers/Keen project).

IV. Methods:

Specific Aim 1 will test the hypothesis that binge-like prenatal alcohol exposure in the 1st-trimester sheep model and the 3-trimester sheep 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.

Specific Aim 2 will 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.

Specific Aim 3 will 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.

Specific Aim 4 will 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.

V. Accomplishments and Results:

Studies to address specific aims 1, 2 and 3 are underway. Four lambs have completed these studies. In addition, eleven lambs have been born and will complete studies within the coming months. Currently, there are thirty pregnant ewes enrolled in the studies. By the end of this breeding season (by the end of February 2009), all subjects necessary to complete specific aims 1, 2 and 3 will be bred and all of these will have completed the studies by the fall of 2009.

To assess facial dysmorphology in the sheep model, we will obtain standardized measures of facial features at three time points for each lamb: at birth, at two months (weaning), and again at five months. We have established 25 facial measurements that we will obtain from each lamb, and we have acquired the calipers (spreading and sliding) necessary for taking these measures. Facial measurements have now been obtained from 13 newborn lambs, 8 two month old lambs and 4 five month old lambs.

We will utilize structural measures (MRI and cell counting in the cerebellum, hippocampus and raphe) and functional measures (eyeblink classical conditioning to assess cerebellar and hippocampal injury and T-maze to assess hippocampal injury). To date, we have collected tissues for structural analysis from 4 subjects (measurements have not yet been completed on these) and have performed in vivo MRI studies on 2 subjects. Eyeblink conditioning and T-

maze training assessments have been completed on four subjects and are ongoing in the postnatal lambs.

The hypothesis of Specific Aim 4 is that choline supplementation provided to pregnant ewes during gestation will ameliorate effects of 3-trimester alcohol exposure on behavioral and brain outcomes. The determination as to when to begin choline supplementation (immediately after mating, or at the beginning of the 2nd trimester), and in conjunction with which exposure model (1st-trimester or 3-trimester) will be informed by results from the rodent component of the CIFASD project; at this time there is no progress to report on Specific Aim 4.

VI. Discussion.

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 the 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 ARND, the sheep model will utilize eyeblink classical conditioning and T-maze learning in subjects for whom the dose and pattern of exposure is precisely known and other environmental conditions are 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 whether dysmorphology is expressed in sheep. At present, there is no ideal animal model of FASD dysmorphology. The developmentof 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 preventive 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, in which the dose and timing of exposure is precisely known.

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 in which the independent measures can be precisely controlled and for which 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. Interrelation with Aims of the Consortium and Other Projects:

The Consortium has six major objectives. The interrelation of this project with the others is outlined below by objective.

1. Establishing standardized diagnostic criteria and methods of assessing FASD.

Almost all of the projects are directly related to this aim. The 3D facial imaging project (T. Foroud, PI) will use novel facial imaging methods to identify features important in FASD. The neurobehavioral project (S. Mattson, PI) will identify behavioral phenotypes relevant to FASD, while the brain imaging project (E. Sowell, PI) will identify morphological and functional brain changes in FASD. These projects interrelate with each other as well as with the Dysmorphology Core to assess the relationship between face, brain, and behavior. The prospective Moscow project (C. Chambers, PI) will utilize careful specification of alcohol exposure and fetal, neonatal, and infant structural and neurobehavioral outcomes. This project interrelates with the developmental prenatal ultrasound project of A. Hull, PI. The basic research project of T. Cudd (PI) utilizing a sheep model of FASD will also assess how differences in duration of prenatal alcohol affect both structural (face and brain) and behavioral outcomes. The mouse model of F. Zhou (PI) will provide an animal model parallel to the facial imaging project of T. Foroud and will examine how timing of alcohol exposure influences the pattern of facial dysmorphology. Facial dysmorphology and brain imaging will also be assessed in the mouse MRI study of K. Sulik (PI). Dr. Sulik's project will also inform the brain imaging project of E. Sowell. The Dysmorphology Core (K. Jones, PI), with support from the Informatics Core (C. Stewart, PI), will play a critical role in integration of the data to develop diagnostic criteria for FASD with greater sensitivity and specificity.

2. Enhanced understanding of the neurobehavioral phenotype of FAS and ARND.

Comprehensive neurobehavioral assessments will be conducted at several sites. The major clinical project involved in this aim is led by S. Mattson, PI. This aim utilizes our previous findings from several projects in developing and refining neurobehavioral profiles for FASD. Children and adolescents from six sites (S. Africa, San Diego, Los Angeles, Atlanta, Northern Plains, New Mexico) will be evaluated. This will involve the projects of P. May and E. Sowell, PIs, as well as a subcontract with C. Coles. Importantly, the specificity of these neurobehavioral profiles will be assessed by comparison to contrast groups. The prospective Moscow project of C. Chambers will assess infants exposed to alcohol prenatally to determine neurobehavioral outcomes as early in life as possible. Several of the animal projects (T. Cudd, F. Zhou, and J. Thomas, PIs) will be utilizing related behavioral outcomes in their projects. Importantly, the neurobehavioral data will be integrated with both brain morphology and facial characteristics.

3. Enhanced understanding of FASD dysmorphology through 2D and 3D image analyses.

During the past 3 years the Facial Imaging Core has obtained digitized images of children with FASD. This Core has now been converted to a research project and this aim will continue, utilizing new camera technology. The previous images and new ones to be obtained from San Diego, Los Angeles, Atlanta, and S. Africa will be utilized to assess changes in facial characteristics in individuals over time, as well as to compare dysmorphic characteristics within and between diverse ethnic groups. Importantly, neonatal images will be taken for the first time from the Moscow and Ukraine project. The Basic Science projects of T. Cudd, F. Zhou, and K. Sulik will utilize morphometric analysis in their animal models, the latter two using similar technology. The Sulik proposal will use high resolution MRI reconstruction to conduct similar analyses to those being done with the 3D images and also with the MRI project of E. Sowell. The identification of novel facial features which discriminate individuals with FAS from other

alcohol-exposed individuals, as well as control subjects will be identified using machine learning approaches and established methods from evolutionary biology.

4. Characterization and correlation of structural and functional neurobehavioral deficits.

Changes in brain morphology and function are being assessed in several projects. Structural brain changes using advanced imaging methods will be examined in a mouse model (K. Sulik, PI) aimed at assessing critical periods of exposure and how this exposure impacts brain development. Similarly, the sheep project of T. Cudd will use structural imaging and quantitative neurohistology to explore brain/behavior alterations following ethanol exposure over different durations. The mouse model of F. Zhou will also examine neuropathological changes associated with prenatal alcohol exposure. These results will inform the human brain project (E. Sowell, PI) and expand our knowledge of how alcohol affects various brain structures and functions. In addition, the developmental prenatal ultrasound project of A. Hull will be pursuing morphometric changes in brain.

5. Early case identification.

Intervention that is enacted early in life has proven to be beneficial in FASD. Therefore, two projects (C. Chambers, PI; A. Hull, PI) will seek to improve our ability to provide early detection of FASD. The developmental project of A. Hull will follow up on data collected in one of our early pilot projects showing the feasibility of using prenatal ultrasound to detect possible FASD. Prenatal ultrasound measures will now be correlated with structural and neurobehavioral features in infants with well documented histories of prenatal alcohol exposure. C. Chambers will evaluate the quantity, frequency, and timing of alcohol use in pregnancy on structural and behavioral outcomes during the neonatal period and infancy. The basic science mouse projects (F. Zhou, PI and K. Sulik, PI) and sheep project (T. Cudd, PI) will help inform this aim as well, as should the clinical neurobehavioral and brain projects. The intervention project of P. May, PI, involves improving outcomes in infants and toddlers and will utilize early case identification methods developed by the other projects.

6. Interventions to reverse or ameliorate neurobiological deficits and in utero therapeutics.

Despite the best intentions and efforts, some women will drink during pregnancy. Thus, it is important to develop interventions to ameliorate the negative outcomes in the offspring. Several projects address this issue. The nutritional intervention projects of C. Keen (human - contained in the Chambers project), J. Thomas (rat developmental project), and T. Cudd (sheep) investigate promising nutritional interventions, including choline supplementation. The basic research project of K. Miller (PI) builds upon previous data showing that ethanol teratogenesis is in part caused by altering the function of the L1 cell adhesion molecule. Future drug design aimed at antagonizing ethanol's action is dependent upon solving the 3D structure of binding sites for ethanol and its antagonists. Importantly, this project will also identify signaling pathways that determine whether L1 expressed in different cells is sensitive or insensitive to ethanol inhibition. The identification of these key signaling elements will permit a targeted search for genetic polymorphisms that underlie differential sensitivity to alcohol teratogenesis in human populations. The F. Zhou (PI) project will also address the development of ethanol antagonists, by continuing work investigating NAP and SAL, two trophic factor analogues shown to be effective in mitigating brain and facial changes in mice following early gestation exposure. The clinical project of P. May (PI) extends previous data collected in the consortium on cognitive control and language and literacy interventions to improve school performance in children with FASD. Additionally, a new early intervention aim of this project will apply social and emotional interventions to mother/infant dyads aimed at improving self-regulation and attachment, as well as language development of the infants.

VIII. Plans for the Next Year:

We plan to complete the experiments necessary to complete Specific Aims 1-3 by the fall of 2009.

IX. Publications:

None

X. Posters and Presentations:

None

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

II. Title of Project: Magnetic Resonance and Diffusion Tensor Imaging of a Mouse FASD Model U01 AA171204

III. Objectives:

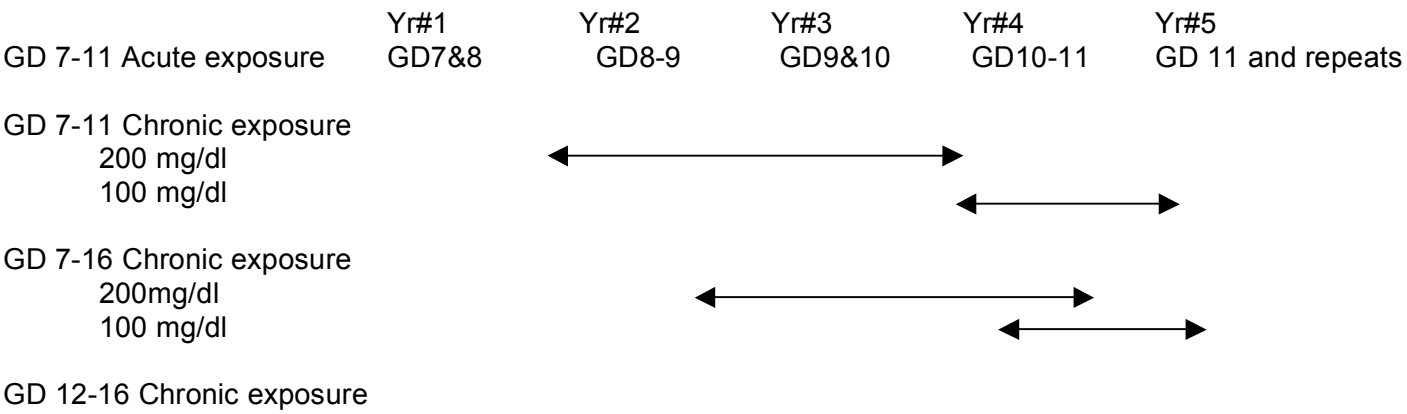
The primary objective of this work is to inform the establishment of improved diagnostic criteria for FASD and to make new discoveries regarding the impact of prenatal ethanol exposure on the brain and face. Utilizing a mouse FASD model, critical periods of ethanol-induced teratogenesis, with emphasis 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.

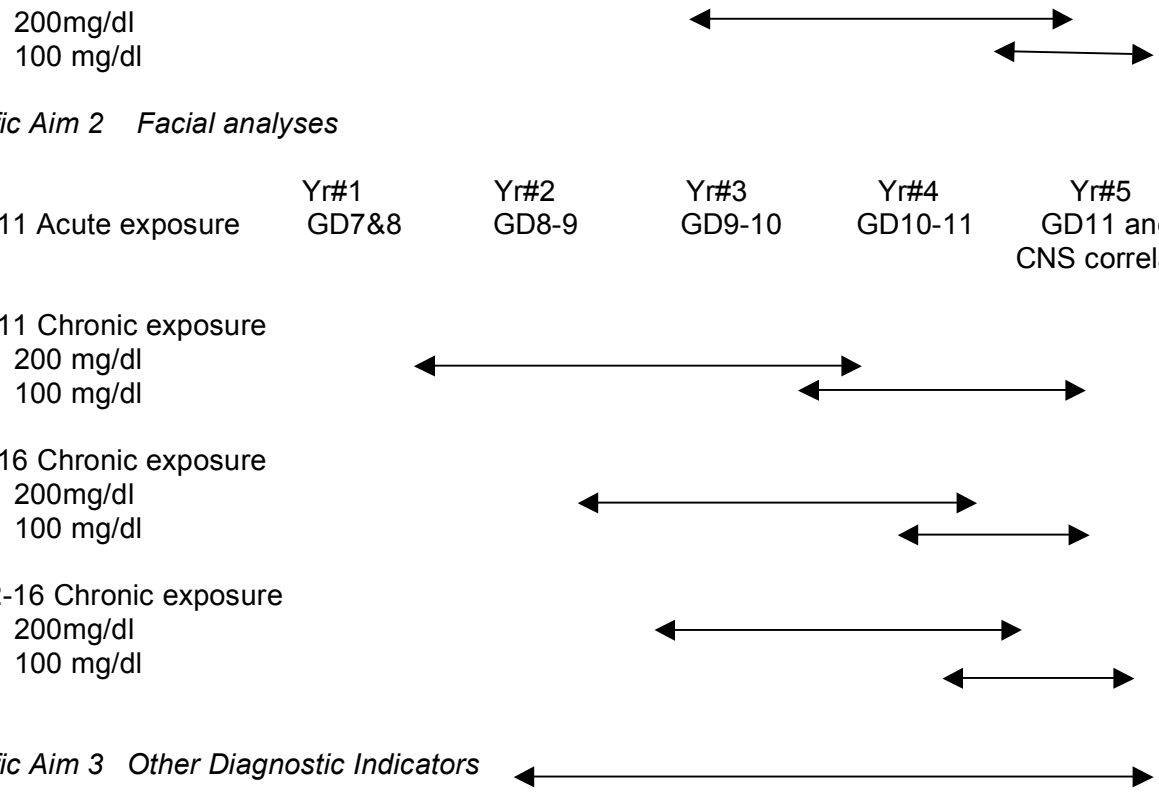
IV. Methods:

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. For Specific Aims 1&2, linear, volume and/or area measurements for a number of selected brain and facial regions will be made, with comparisons made between relevant control and alcohol-exposed specimens and potential correlations between facial and brain dysmorphology identified. For Specific Aim 3, regions other than the brain or face that may serve as diagnostic indicators of prenatal ethanol exposure will be similarly investigated.

Timeline for the investigations (as originally proposed):

Specific Aim 1 Brain analyses





V. Accomplishments and Results:

Overview

In the first 1.5 yr period since this project was funded, our accomplishments have exceeded our expectations as set forth in the proposed timeline shown above. Most notably, we have completed the MRI scans and nearly all of the segmentation work for the acute exposure studies outlined in Specific Aim #1. That is, we have what we feel is adequate MRI data to define the stage-specific CNS phenotype for each of alcohol exposure days 7-11 of gestation (GD7-11). Our first paper details the GD8 findings and has been accepted with revisions for publication in Alcoholism Clinical and Experimental Research. We are now analyzing and writing up the data for the other time points. In addition, we have completed the MRI scans of the 200mg/kg GD 7-16 chronic exposure group (supplied by the Zhou lab)and have begun segmenting them. Regarding the proposed DTI work, we have successfully applied this technique and have acquired preliminary data for the GD 7 exposure group. For Specific Aim 2, while MRI scans (for the groups indicated above) have been collected, to date they have not yet been quantitatively analyzed. For Specific Aim 3, we have been successful in identifying dysmorphology of the nasal cavity as a potential FASD diagnostic indicator. This is presented and discussed in our submitted (GD8-exposure) manuscript. Also, using the MRI data, we have begun to examine the hearts of selected specimens. Notably, we have scanned more fetuses than initially proposed. This has been financially possible with the aid of the Duke Center for In Vivo Microscopy.

In addition to the above, we have conducted studies that are essential in assuring the validity of our conclusions. We have shown differing rates of early development and differing sensitivities to alcohol-induced dysmorphogenesis in two different C57Bl mouse substrains (6J vs 6N) and determined that for consistency, only the C57Bl/6J substrain will be utilized for this investigation. Additionally, recognizing that prenatal alcohol exposure yields developmental delay, we have used MRI to analyze the brains of control

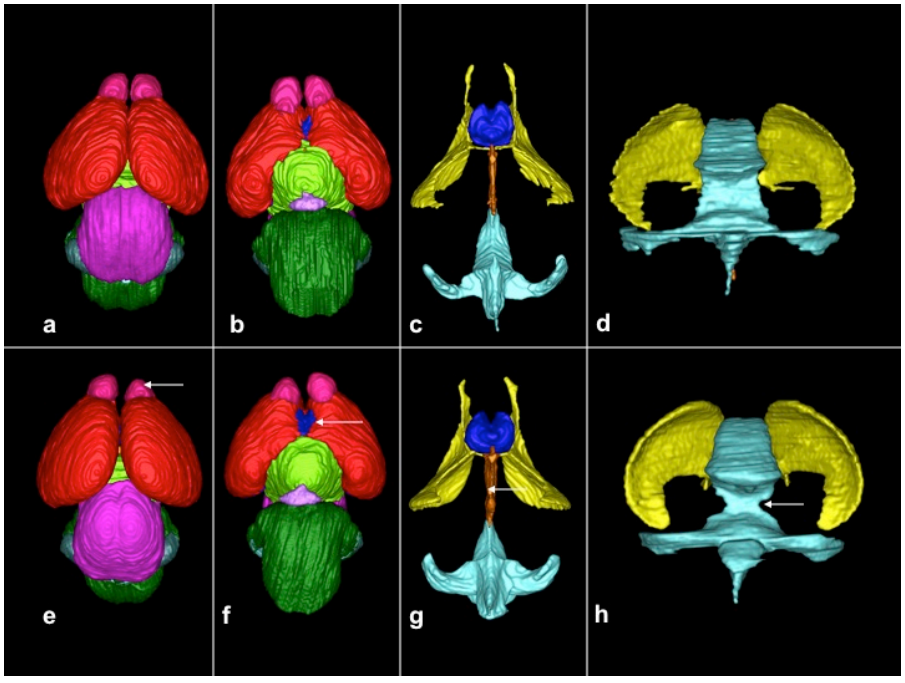
fetuses that are chronologically a full to a half of a day younger than our ethanol-exposed GD17 fetuses. Availability of this data allows appropriate comparisons to be made.

Progress (since June, 2008)

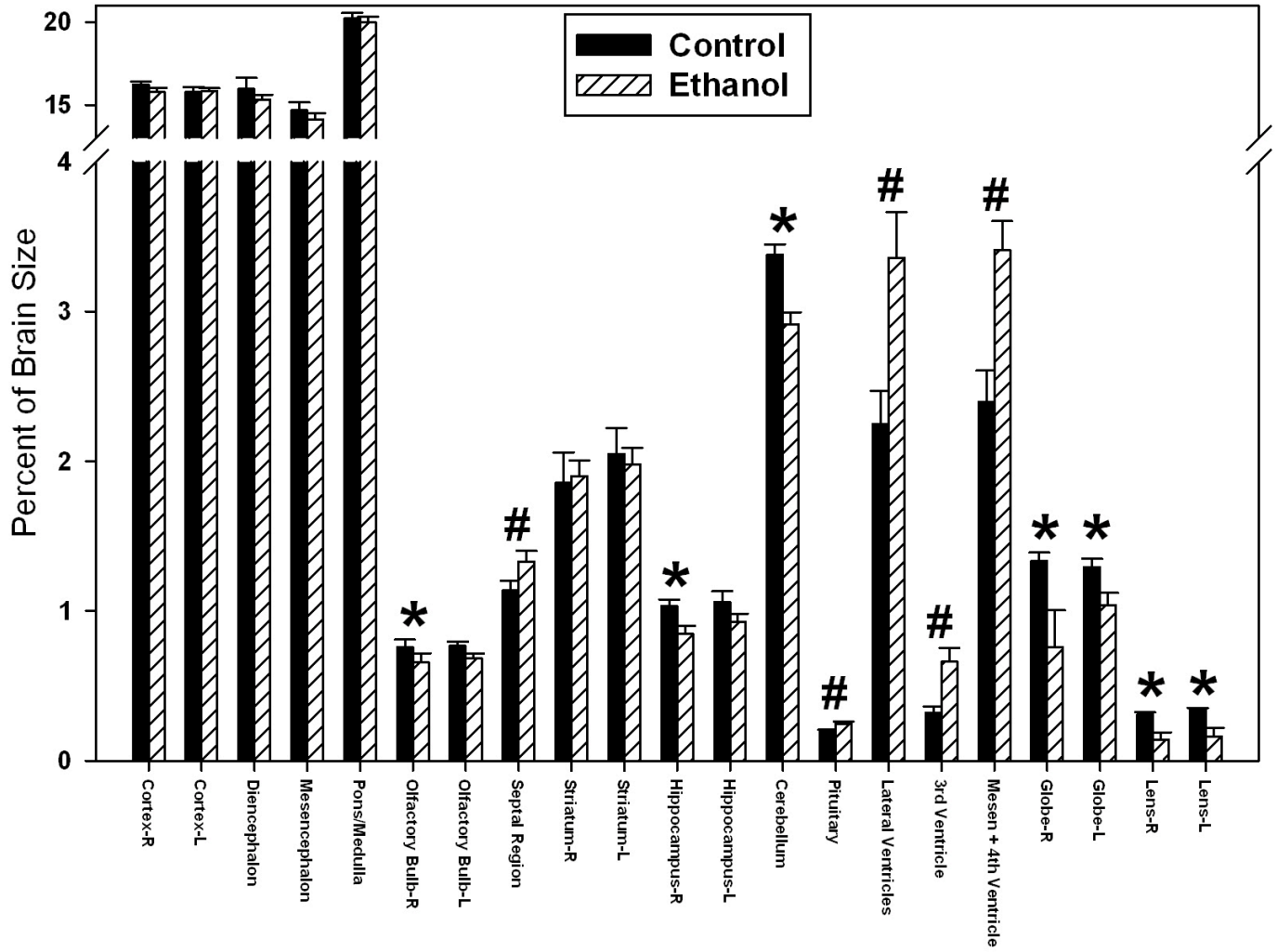
1. The results of our work showing that linear measures for GD7 & 8 exposures illustrated reduced frontothalamic distances and the consistency of these findings with those from ultrasound studies as described by AD Hull and co-workers (this consortium) were presented at the 2008 David W. Smith Workshop on Malformations and Morphogenesis (*see attached* abstract entitled "High resolution magnetic resonance imaging of alcohol-exposed fetal mice confirms and informs human prenatal ultrasound studies").

2. Dr. Shonagh O'Leary-Moore, a postdoctoral fellow in our laboratory, described her DTI work to date as a FAST data presentation at the 2008 FASDSG study group meeting (*see attached* abstract entitled "Diffusion tensor imaging reveals fiber tract abnormalities in alcohol-exposed fetal mouse brains).

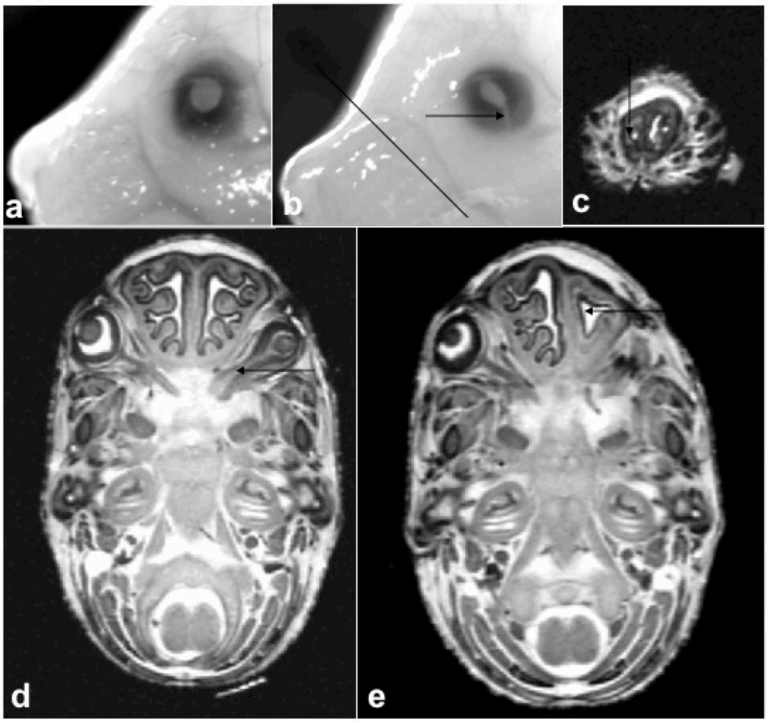
3. Dr. Scott Parnell, a postdoctoral fellow in our laboratory, has submitted for publication our first MRI manuscript entitled "High resolution magnetic resonance microscopy defines ethanol-induced brain abnormalities in fetal mice: effects of acute insult on gestational day 8" (*see attached*). As illustrated in the color figure below, as compared to the images of a normal brain (a-d), those from a fetus that had been acutely exposed to ethanol on GD8 (e-f) illustrate a visibly small right olfactory bulb (arrow in e) and excessive space between the bulbs, a relatively large ventral midline view of the septal region (arrow in f); notable enlargement of the third ventricle (arrow in g) as observed from a ventral view of the reconstructed ventricular system; and narrowing of the cerebral aqueduct (arrow in h) as observed in a posterior view of the ventricular system. Color-coding is as follows: red = cerebral cortex; dark pink = olfactory bulbs; magenta = mesencephalon; light green = diencephalon; dark green = pons/medulla (rhombencepalon minus cerebellum); teal = cerebellum; dark blue = septal region; light purple = pituitary; yellow = lateral ventricles; orange = third ventricle; light blue = mesencephalic and fourth ventricle.



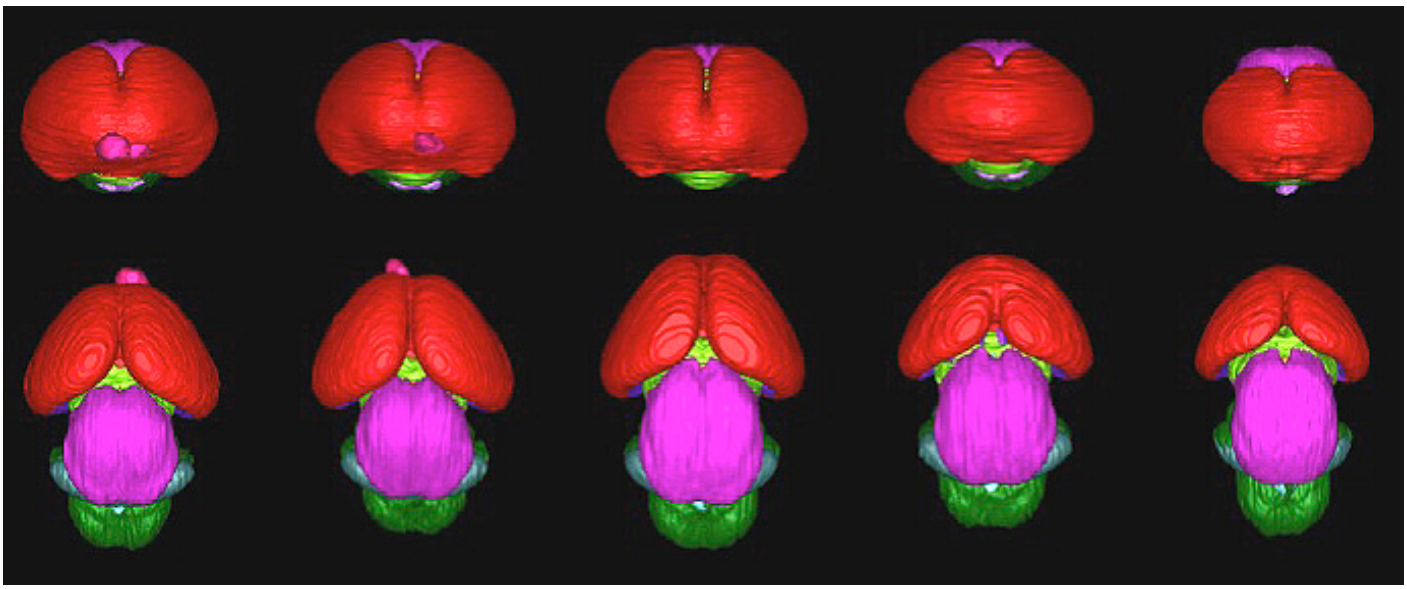
Graphic illustration of ethanol-induced proportional volume changes in GD 17 fetal mouse brain regions is provided below. Significant average volume reductions are indicated by (*), while increases are indicated by (#).



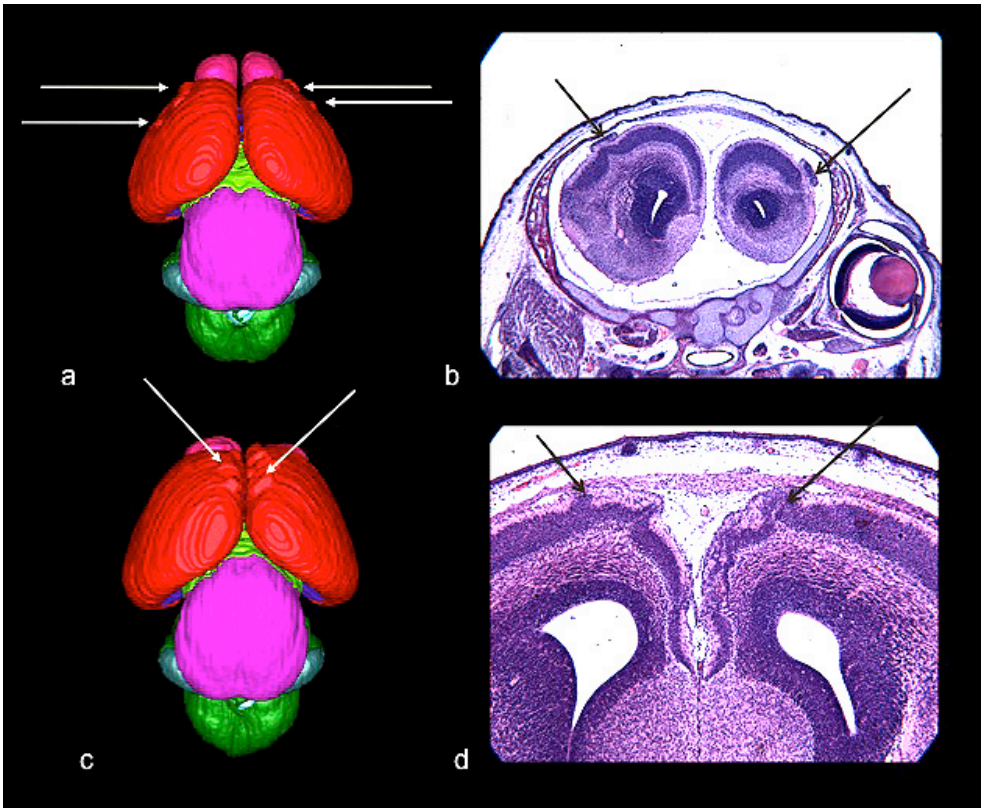
In addition to the above, optic abnormalities and choanal stenosis were documented in alcohol-exposed fetuses. As shown in the figure below, microphthalmia and irideo-retinal coloboma (arrow in b) are evident in GD 17 ethanol-exposed fetuses. [Compare the affected left eye to the normal left eye of a control GD 17 fetus in (a).] Additionally, MRI revealed an optic nerve coloboma in the right eye of an ethanol-exposed fetus (arrow in d). and a coronal MRI scan (c) at the level indicated by the line in (b) illustrates a pinhole - like opening (arrow), representing the extreme narrowing of the right choana. A horizontal scan (e) of the fetus in (c) illustrates the simple contours of the small right nasal cavity (arrow).



4. Elizabeth Myers Godin, a Ph.D. candidate in our laboratory, is in the process of preparing a manuscript describing the results of her MRI analyses of GD7-exposed fetuses. As expected, GD 7 exposure resulted in a spectrum of CNS abnormalities, with a major site of deficiency being the rostral and ventro-medial forebrain. Some of the severely affected animals had CNS dysmorphology that is consistent with lobar (left 3 specimens in the image below) or semilobar (right 2 specimens pictured below) holoprosencephaly.



Of particular interest, other GD7-exposed animals presented with cerebral cortical dysplasia/heterotopias. One of these animals had a relatively normal-appearing brain otherwise (a,b). The cerebral cortical abnormalities in another fetus (c,d), in which the olfactory bulbs were abnormal, were confined bilateraly to the medial region, including the cingulate gyri.



5. We have completed studies with the Zhou group assessing differences between ethanol's teratogenesis in C57Bl/6J versus 6N mice. The 6J substrain was found to be more sensitive and has been adopted for the planned joint experiments. An abstract describing this work is being prepared for 2009 RSA submission.

6. We have collected MRI scans from the dietary GD7-16 ethanol exposure group and will begin segmentation and analyses in the near future.

7. Recognizing that our ethanol exposure paradigm results in a generalized developmental delay, in addition to examination of GD 17 control fetuses, Dr. Shonagh O'Leary Moore has conducted MRI analyses of GD16 and 16.5 fetal mouse brains. The results of this work has supported our conclusion that ventricular enlargement observed in the ethanol-exposed fetuses is real and not merely a result of developmental delay. Along with her GD 10 and 11 exposure analyses, Shonagh is preparing this data for publication.

8. We have initiated study of heart morphology in 3D reconstructed MRI scans. Focus is currently on the GD 8 and 11 exposure times.

VI. Discussion:

Our imaging studies are providing important new data from which to draw for the study of human FASD subjects and to further our understanding of the range and critical periods for induction of defects that result from prenatal alcohol exposure. Progress during this 6 month period has been highlighted by discovery of cortical heterotopias resulting from acute GD 7 ethanol exposure. Heterotopias have been reported in autopsy studies of human FAS. Also, they are a significant finding in human holoprosencephaly and are associated with seizure activity. Noteworthy is that in one of the affected animals, the involved cortex included the cingulate. This is also a region that has been of particular interest in FASD. Since the embryos are so young (the neural plate is not yet morphologicaly distinct) at the time in our mouse model during which the insult that caused the cortical heterotopias occurred (GD7), it will be a challenge to determine the underlying pathogenic sequence of events.

As confirmed and extended by our GD 8 ethanol exposure-group analyses, the similarity between the ethanol-induced teratogenic endpoints and the features of the CHARGE association (some of which are shared with DiGeorge) is striking. Recognition of these features as part of FASD promises to aid in both pre- and postnatal diagnosis. Additionally, knowledge of clinical findings in CHARGE, should aid in guiding future clinical and basic FASD research.

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 will compliments that employing 3D images as are being prepared and analyzed by Dr. Faroud'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 6 Months:

During the remainder of this funding period, we hope to:
1. Complete our analyses and submit manuscripts describing our MRI results from the acute GD 7, 9, 10 and 11 exposure groups and submit abstracts for the 2009 RSA meeting
2. Complete the segmentations and analyses of the chronic GD 7-16 200mg/dl exposure group.
3. Begin quantitative analyses of facial dysmorphology.
4. Begin to collect the GD 7- 11 200mg/dl- chronic exposure group fetuses.
5. Continue to segment hearts from control and experimental fetuses.

IX. Publications:

Parnell SE, O'Leary-Moore SK, Myers EA, Dehart DB, Johnson GA, Styner MA, Sulik KK, High resolution magnetic resonance imaging defines ethanol-induced brain abnormalities in fetal mice: effects of acute insult on gestational day 8. (Full paper, submitted, 2008; under revision)

Parnell SE, Johnson GA, Sulik KK, Brain Parnell SE, Johnson GA, Sulik KK, Brain abnormalities resulting from gestational day nine ethanol exposure in the mouse: a study utilizing high resolution MRI, Alcohol Clin Exp Res, 32:204A 2008

Sulik KK, O'Leary-Moore SK, Parnell SE, Myers EA, Dehart DB, Johnson GA, Chambers CD, and Hull AD,

High resolution magnetic resonance imaging of alcohol-exposed fetal mice confirms and informs human prenatal ultrasound studies. Proc of the Greenwood Genetics Center (Abst. in press)

In preparation:

Sulik KK, O'Leary-Moore SK, Godin E, and Parnell S, Prenatal neurodevelopment and effects of teratogenic agents, In: Drugs in Pregnancy –The Price for the Child, Preece and Riley (Eds.), in prep 2008-9

2009 RSA abstracts (3)

X. Posters and Presentations:

Presentations:

S. O'Leary-Moore, FASt data session, FASDSG, RSA (June, 2008)
K. Sulik, Smith Conference (August, 2008)
K. Sulik, UNC Reproductive Epidemiology Course lecture (August, 2008)
K. Sulik, Carolinas Conference (Oct, 2008)
K. Sulik, UNC Psychiatry Dept, Neurobiology of Mental Illness lecture (Nov, 2008)
E. Myers Godin, UNC Toxicology Dept. Seminar (Dec, 2008)

I. Principal Investigator: Feng C. Zhou, Ph.D.

II. Title of Project: Mouse Model Neuro-Facial Dysmorphology: Translational and Treatment Studies UO1 AA14819

III. Objectives:

Specific Aim 1.Determine the longitudinal 3D-facial dysmorphology as a function of the dose and developmental timing of alcohol exposure via a liquid diet consumption in the C57BL/6 mouse model.

Specific Aim 2.Determine the brain-structural and neuro-facial abnormalities as a function of the dose and developmental stage of alcohol exposure. (in parallel with Aim 1)

Specific Aim 3.Determine the extent to which neurotrophic peptide NAP/SAL, given concurrently with prenatal alcohol exposure, will provide long-term protection against alcohol-induced neuro-facial dysmorphology and neurobehavioral deficits.

IV. Methods:

C57BL/6 mice (of Harlan and Jackson vendors) female mice were provided chow or liquid diet containing 2.4% (v/v) ethanol for two days to achieve acclimation; and then increased to 4.8% (v/v) for 5 consecutive days. After this acclimation/acquisition period, the mice were taken off the liquid diet for an abstinence period, during which they were maintained on laboratory chow and water while they recover from any potential alcohol withdrawal symptoms. This alcohol deprivation period averages 14-16 days. During this time, the mice were bred. Ethanol 4.8% (v/v) in the liquid diet were re-introduced to the experimental group of pregnant mice on GD7. They were maintained on this diet GD7-16. All pair-fed control groups (with isocaloric maltose-dextrin substituted for ethanol) were pair-fed equal amount of diet during the target gestational period isocaloric with 4.8%(v/v) group (ensuring nutritional equality across all liquid diet groups). The postnatal pups The E17 embryos were obtained from these dams for microvideo of facial anthropometry, MRI/DTI (Sulik), and the microCT (Y. Liang) analyses.

V. Accomplishments and Results:

1. *Alcohol drinking program and preliminary alcohol effect in E17 C57BL/6 (B6) mice*
We have reported the drinking score for both stocks of B6 mice drank over 20g/kg of alcohol per day with the Harlan line (24.6±1.6 / 22.8±1.5 g/kg, pre- /during-pregnancy) greater than the Jackson line (21.8±1.3 /20.6±1.6 g/kg). The delay in fetal development is noted below.

Fetal body weight: body weights were reduced in both lines: Harlan, Control 0.53±0.03g, Alcohol 0.45±0.02g; Jackson, Control 0.55±0.03g, Alcohol 0.48±0.03g.

Initial Anthropometry: The 2-D Anthropometric measurements were selected as analogues to humans. Alcohol affected both Jackson and Harlen lines (as compared to their correspondent PF and Chow) in Whisker pad width, Bitragal length, Nasal bridge, left upper face distance and left midface distance (ANOVA and Student T-test; $P<0.05$). Some similar reductions in PF and alcohol treatments as compare with Chow controls were also observed (e.g. Nasal Width and right Palpebral Fissure in Harlan line).

2. *Computational Facial Feature Analysis for Micro-Video Images*
Two new pieces of computation work have been accomplished since the last report:

A. A multi-angle facial image analysis algorithm has now been developed and implemented. A preliminary analysis was done using 64 mouse embryos, each with multiple facial images taken with a high resolution camera. The analysis results are encouraging, with an 89% classification rate. To facilitate human-mouse comparisons, we are planning to apply the same approach to human faces, and are developing an intuitive feature visualization technique to better compare key facial features for FAS classification between mouse and human.

A paper based on the design on multi-angle facial image analysis has been accepted for publication in the Proceedings of 2009 ACM Symposium on Applied Computing (Pub1). (*n.b.: this is a full peer reviewed and archived publication. These type of publications are treated the same as regular journal papers in Computer Science as well as most Engineering disciplines).*

B. 2D to 3D measurement: Using a 2D to 3D reconstruction technique, 3D distance measurements were reconstructed using landmark points placed on 2D images of different angles. Our tests show that the 3D measurements obtained using this technique has an accuracy with a statistical error around 5%. This would allow 3D morphometric analysis of mouse embryo data.

3. *MicroCT imaging to evaluate craniofacial bone dysmorphology*

C57BL/6 from Jackson Lab were treated with 4.8%v/v alcohol prepregancy and at E7-16 (ALC-ALC), and compared with Chow, Pair-fed (PF-PF), and pre-preganancy alcohol control (ALC-PF). Surrogate fostering with a normal dame is practiced. Longitudinal MicroCT imaging has been obtained from offspring at postnatal 7 day (P7) and again at P21 age.

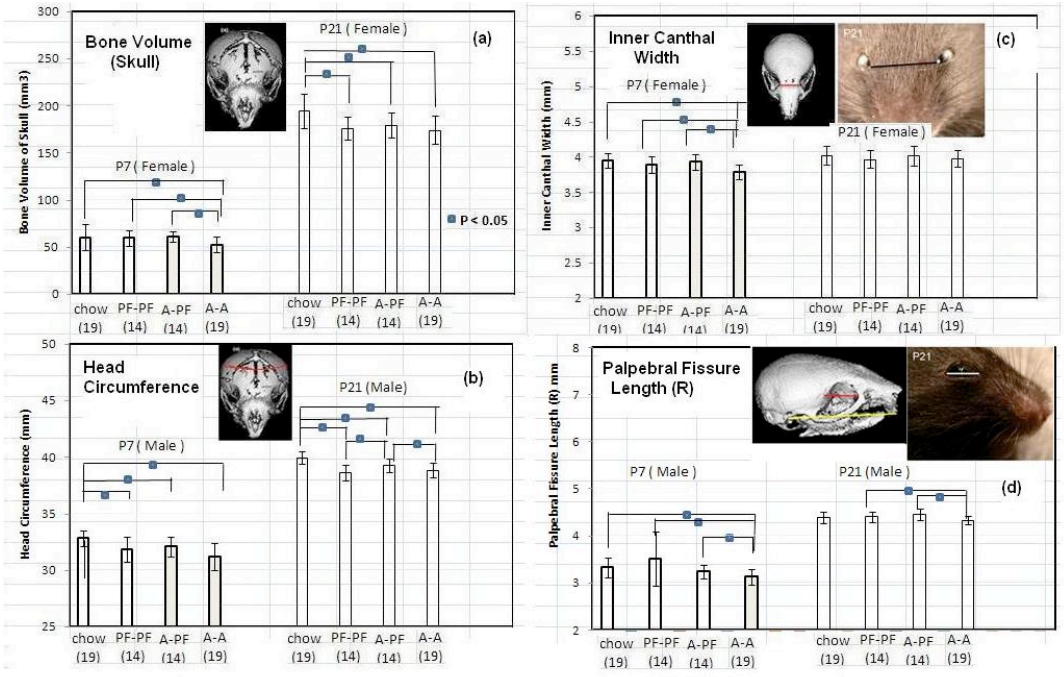


Figure 1. Effect of alcohol treatment on the craniofacial bone measurements (*P<0.05, paired t-test). (a)The female bone skull volume is smaller in ALC-ALC than the others at P7 and P21. (b) Alcohol reduced head circumferences at P7, and partially at P21. (c) Smaller Inner canthal width at P7 but not at P21. (d) Shorter right ocular diameter (comparable to palpebral fissure length) at both P7 and P21. A-PF (ALC-PF); A-A (ALC-ALC)

A. *Cranial Bone volumes:* Alcohol affected Bone volumes of the skulls as indicated in P7 disregard feeding pattern (Figure 1). Nutrition is likely another factor as indicated at a later stage on P21. This indicates that prenatal alcohol and nutrition are both critical factors in long-term development of skull bone volume.

B. *Cranial anthropometry*: The skull anthropometric measurements were performed to a better understanding facial anthropometry. Among craniofacial anthropometric landmarks which have been measured, the head circumference, the inter-orbital distance (between the eye sockets), and ocular diameter (comparable to palpebral fissure) were found to be significantly reduced by alcohol (Figure 1). There are also indications that some landmarks/features manifest differently at various developmental stages. For instance, the inner-canthal width was smaller in ALC-ALC than all other groups at P7 ($P < 0.05$) but the difference caught up at P21. (See Figure 1c). The current results represent one third of the data collected for this testament (E17). Upon the completion of all data analysis, correlation with microvideo facial measurements is to be pursued.

4. *Reduced development of 5-HT neurons and NAP and SAL treatment*
Alcohol induced reduction of 5-HT neurons and their protections by NAP and SAL treatment is now published (Pub. 2).

VI. Discussion:

1. The findings of 2-D facial imaging anthropometry point to the fact that facial development is a major target for high alcohol exposure at the second trimester. Multiple changes in the size of facial development were found in both lines, Harlan and Jackson. The finding also indicated that alcohol induced facial dysmorphology is prominent at the embryonic stage.

2. With the same treatment paradigm, but analyzed in more mature age, the MicroCT analysis indicates that bone volume are reduced. This may be an alcohol induced metabolic change leading to reduction of calcium deposit or bone cell formation. Craniofacial bone change may underlie some anthropometric facial dysmorphology (e.g. ocular diameter for palpebralfissue) and can be strong supporting evidence for identifying novel dysmorphic features. Furthermore, some facial dysmorphology may develop over time from the embryonic stage to the postnatal (e.g. inner canthal width).

3. The pair-fed effect indicated that the nutrition disparity can be a culprit in alcohol induced facial dysmorphology or craniofacial developmental delay. This would also suggest that nutritional supplement during or after alcohol exposure may ameliorate the dysmorphogenesis in FASD.

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

A cross-species anthropometry analysis chart including mouse, sheep, and human were discussed, in which the comparable parameter will be analyzed. We have obtained a preliminary data for mice, which will be used for comparison. We have also completed a group embryos of E7-16 chronic treatment to Dr. Sulik for MRI /DTI analysis to compare with her other groups with single-day treatment.

VIII. Plans for the Next Year:

1. Continue facial anthropometry analysis beyond the embryonic age to include postnatal day 1.
2. Continue acquisition of 3D MicroCT image data and full analysis of anthropometry and bone features.
3. The 3-D measurement of multi-angle Microvideo facial image will begin. This will allow for more comparable comparisons with human facial imaging.

IX. Publications

1. Shiaofen Fang, Ying Liu, Jeffrey Huang, Sophia Vinci-Booher, Bruce Anthony, and Feng Zhou. Alchohol Exposure Analysis of Mouse Embryos Using Multi-angle Facial Image Analysis. Proc. of Association for Computing Machinery (ACM) Symposium on Applied Computing, to appear, 2009.
2. FC. Zhou, Y Fang, C Goodlett, Peptidergic Agonists of Activity-Dependent Neurotrophic Factor Protect Against Prenatal Alcohol-Induced Neural Tube Defects and Serotonin Neuron Loss, Alcoholism : Clinic & Exp. Res, 2008 Aug;32(8):1361-71.

Manuscript in preparation:

1. Y. Liang, H, Ai, B. Anthony, and F. C. Zhou, MicroCT analysis of craniofacial dysmorphology after alcohol exposure in c57bl/6 mouse model". To be submitted.
2. B. Anthony,Y. Liang, C. Goodlett and F. C. Zhou, Facial Morphometric Analysis of C57Bl/6 Mouse Models of Fetal Alcohol Spectrum Disorder.

X. Posters and Presentations:

1. F. C. Zhou, S. Fang, B. Anthony, J. Rodriguez[2] Using 3D facial image analysis for diagnosis of fetal alcohol syndrome in mice.
2. Liang y, Ai, h, Anthony BC, Zhou FC, A, "micro-ct imaging of facial and cranial bone dysmorphology induced by alcohol exposure in a c57bl/6 mouse model". 2008 RSA/ISBRA Joint meeting, June 28 - July 2. Washington, D.C, 2008.

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

II. Title of Project: Spectrum of and Nutritional Risk Factors for FASD in Russia and Ukraine U01 AA014835

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. Summary of Accomplishments and Results:

1. Approximately 90 new study subjects have been recruited, blood samples collected and repeated measure 2D ultrasounds plus additional measures incorporated into Dr. Hulls's developmental project have been performed.
2. Assignment to micronutrient supplementation according the randomization scheme is proceeding as planned, and subjects report taking the multimicronutrient supplements with or without choline supplementation.
3. Training of the study psychologists and the neurophysiologist at the Ukraine site has been completed as of November, 2008, by Dr. Coles and Dr. Kable.
4. Interview data from 210 subjects has been reviewed, cleaned and is appropriate for uploading to the central repository.
5. Appropriate institutional agreement has been prepared by Ukrainian collaborators for Dr. Foroud to allow for customs import of 3D camera for use between March, 2009 and September, 2009.
6. IRB amendment has been submitted to permit collection of maternal hair samples for banking.

7. Manuscript on paternal relationship variables and continued binge drinking in collaboration with Sharon Wilsnack is in preparation.
8. Manuscript regarding preliminary data on baseline nutritional status is in preparation.
9. Initial nutritional analyses have been completed on 49 blood samples in Dr. Keen's lab.
10. Specific Aim 2A – to assess the relation of baseline nutritional status to alcohol exposure group has been addressed.

V. Discussion:

Nutritional Analyses:

During the period under review, Dr. Keen's lab has worked to further optimize HPLC conditions to simultaneously determine not only plasma Vitamin A and Vitamin E (α –tocopherol), but also γ-tocopherol and select carotenoids. The solvent system employed consists of a mixture of acetonitrile (80%), methanol (10%), and dichloromethane (10%), run isocratically at a flow rate of 0.9 mL with a column temperature at 25 °C. Retinol and retinyl acetate are monitored at 325 nm, tocopherols are measured at a wavelength of 290 nm, and carotenoids are detected at a wavelength of 450 nm. Reversed phase (RP-) HPLC separation is carried out on an Agilent 1100 system with solvent degasser, binary pump, temperature controlled autosampler, temperature-controlled column compartment, and a diode-array detector (DAD) monitoring 290 nm, 325 nm, and 450 nm. Separation is achieved using a Luna C18(2) column, 150x4.6 mm, 3 µ particle size with guard cartridge system (Phenomenex, Torrance, CA). This method allows for the simultaneous detection of retinol, retinyl acetate, α-tocopherol, γ-tocopherol, lutein, lycopene, α-carotene, and β-carotene. These new developments will allow us to do a more comprehensive evaluation of the women in the study using relatively small plasma volumes. Importantly, these methodologies will also be of value in assessing the nutritional status of the infants in future studies.

Dr. Keen's lab has received and analyzed 49 samples from pregnant women residing in Rivne Oblast (Table 1). We anticipate that we will now be receiving approximately 80 new samples every two months. With respect to the samples obtained to date, the numbers of women that were assigned to the 6 treatment groups are shown in Table 1. The plasmas that we analyzed represent baseline blood draws for the women. Plasmas were analyzed for Vitamins (Vitamin A retinol, Vitamin E α-tocopherol, γ-tocopherol), carotenoids (β -carotene, α-carotene, lycopene, lutein), minerals (zinc, copper, iron, magnesium, and calcium), choline and choline metabolites (betaine and dimethylglycine), ceruloplasmin activity, TRAP (total antioxidant potential of plasma) and TBARs (thiobarbituric acid reactive substances, an index of lipid peroxidation).

Table 1

Control, No Vitamin	Control + Vitamin	Control + Vitamin + Choline
N=11	N=6	N=4
Ethanol exposed, No Vitamin	Ethanol exposed + Vitamin	Ethanol exposed + Vitamin + Choline
N=12	N=10	N=6

While we consider the data still preliminary in nature we have a number of observations which we find encouraging. One, consistent with the initial hypothesis, low plasma zinc and copper levels are being observed in ethanol-exposed women compared to controls. This finding in the new samples is consistent with previous findings in samples collected at the Russian site, and strongly supports the concept that suboptimal zinc and copper delivery to the conceptus can contribute to developmental abnormalities in women who have high levels of alcohol intake. As the toxicity of alcohol is thought to be due, in part, to free radical-induced oxidative damage, deficits of zinc and copper would be predicted to increase the sensitivity of the developing conceptus to alcohol, given that these nutrients contribute to the oxidative defense system. The activity of ceruloplasmin, a copper binding protein with putative antioxidant properties, was also lower in ethanol-exposed women compared to unexposed controls (See Table 2).

All other parameters measured in these baseline samples were similar between ethanol-exposed and unexposed women. Baseline choline levels were also similar among the groups suggesting that alcohol per se does not decrease choline concentrations in plasma, at least at the varied levels of consumption reported by women in this sample. The effects of micronutrient supplementation with or without additional choline supplementation on plasma metabolites and nutrients will be assessed in future samples and correlated to data on infant growth, structural features, neurobehavioral performance and maternal and pregnancy outcomes.

Regression analyses including all groups show that as weeks of gestation increase, plasma zinc, iron, magnesium, Vitamin A and betaine decrease whereas plasma copper, TBARs, Vitamin E and lutein increase. Plasma copper was highly correlated with plasma ceruloplasmin activity ($P < 0.0001$, $R^2 = 0.737$). Zinc, ceruloplasmin and choline have antioxidant properties. Plasma zinc was negatively correlated with TBARs, an index of lipid peroxidation ($P<0.004$, $R^2=0.170$), and plasma ceruloplasmin activity was positively correlated with TRAP, an index of the antioxidant capacity of plasma ($P<0.004$, $R^2=0.167$). Plasma choline tended to be positively correlated with TRAP ($P=0.06$). Correlations were also noted between plasma choline and dimethylglycine ($P=0.04$, $R^2= 0.084$), and plasma choline and betaine ($P=0.03$, $R^2=0.095$) (See Tables 3-6 and regression results below). Given the robust nature of some of these correlations, we anticipate that we will be able to dissect out group differences in these relationships when the full data sets are in.

Table 2: Analysis by Alcohol

	Unexposed Control	Ethanol Exposed	P value
N	21	28	
TRAP (μM Trolox Equivalents)	170.9 ± 6.9	157.9 ± 6.4	0.1754
TBARs (μM MDA)*	1.14 ± 0.07	1.07 ± 0.07	0.5002
Cp activity (Units/L)	237.0 ± 10.1[a]	210.2 ± 8.6[b]	**0.0485**
Zn (μg/mL)	0.64 ± 0.02[a]	0.57 ± 0.02[b]	**0.0022**
Cu (μg/mL)	1.90 ± 0.06[a]	1.73 ± 0.05[b]	**0.0427**
Fe (μg/mL) *	0.82 ± 0.05	0.78 ± 0.06	0.6365
Mg (μg/mL)	16.21 ± 0.21	15.86 ± 0.17	0.1874
Ca (μg/mL)	83.53 ± 0.66	83.38 ± 1.15	0.9151
Vitamin A retinol (μg/mL)	0.68 ± 0.04	0.75 ± 0.04	0.2758
Vitamin E α-tocopherol (μg/mL)	29.33 ± 1.16	31.29 ± 1.44	0.3186
Choline (μM)	7.20 ± 0.37	8.03 ± 0.28	0.0739
Dimethylglycine (μM)	1.41 ± 0.09	1.46 ± 0.12	0.7575
Betaine (μM)	12.32 ± 0.76	12.82 ± 0.55	0.5882
Lutein (μg/mL)	0.79 ± 0.06	0.75 ± 0.06	0.6110
Beta-carotene (μg/mL)*	0.12 ± 0.01	0.12 ± 0.01	0.3378
Lycopene (μg/mL)*	0.04 ± 0.01	0.04 ± 0.01	0.5613

*For TBARs analysis, N=20 for Unexposed Control group
*For Fe analysis, N=26 for Ethanol exposed group
*For β-carotene, N=18 for Unexposed Control group, N=21 for Ethanol exposed group
*For Lycopene, N=15 for Unexposed Control group, N=19 for Ethanol exposed group

Table 3: Analysis by Treatment

	Control, no Vit	Control + Vit	Control + Vit + choline	Ethanol, No Vit	Ethanol + Vit	Ethanol + Vit + choline	P value
N	11	6	4	12	10	6	
TRAP (μM Trolox Equivalents)	173.9 ± 10.5	174.3 ± 13.0	157.8 ± 13.0	163.6 ± 12.6	157.3 ± 7.3	147.3 ± 11.2	0.6267
TBARs (μM MDA) *	1.10 ± 0.10	1.28 ± 0.13	1.07 ± 0.07	1.07 ± 0.11	1.26 ± 0.07	0.77 ± 0.19	0.0971
Cp activity (Units/L)	229.3 ± 15.4	253.1 ± 16.4	234.2 ± 23.3	219.4 ± 12.2	214.4 ± 13.8	185.0 ± 21.6	0.2123
Zn (μg/mL)	0.64 ± 0.02^{ab}	0.63 ± 0.01^{ab}	0.68 ± 0.05^{a}	0.57 ± 0.03^{bc}	0.54 ± 0.02^{c}	0.62 ± 0.04^{abc}	**0.0153**
Cu (μg/mL)	1.89 ± 0.08^{a}	2.01 ± 0.08^{a}	1.74 ± 0.16^{ab}	1.79 ± 0.08^{a}	1.81 ± 0.07^{a}	1.51 ± 0.13^{b}	**0.0388**
Fe (μg/mL) *	0.78 ± 0.10	0.87 ± 0.06	0.85 ± 0.08	0.73 ± 0.10	0.72 ±0.10	0.94 ± 0.16	0.6996
Mg (μg/mL)	15.94 ± 0.23	16.92 ± 0.45	15.89 ± 0.41	15.80 ± 0.22	15.85 ± 0.32	15.97 ± 0.44	0.2297
Ca (μg/mL)	82.29 ± 0.82	85.54 ± 1.22	83.94 ± 1.30	84.10 ± 1.97	83.65 ± 2.09	81.49 ± 1.53	0.7425
Vitamin A retinol (μg/mL)	0.70 ± 0.07	0.67 ± 0.05	0.68 ± 0.07	0.79 ± 0.07	0.66 ± 0.06	0.82 ± 0.08	0.4795
Vitamin E α-tocopherol (μg/mL)	27.22 ± 1.57	29.77 ± 1.63	34.46 ± 2.14	31.19 ± 2.01	33.43 ± 2.12	27.90 ± 4.15	0.2204
Choline (μM)	7.22 ± 0.52	7.94 ± 0.66	6.03 ± 0.60	8.53 ± 0.47	7.29 ± 0.45	8.28 ± 0.40	0.0672
Dimethylglycine (μM)	1.40 ± 0.16	1.55 ± 0.11	1.24 ± 0.12	1.30 ± 0.21	1.53 ± 0.12	1.67 ± 0.31	0.7278
Betaine (μM)	12.45 ± 1.18	12.52 ± 1.39	11.68 ± 1.63	12.85 ± 0.53	11.71 ± 1.10	14.63 ± 1.35	0.6115
Lutein (μg/mL)	0.72 ± 0.08	1.05 ± 0.12	0.62 ± 0.07	0.75 ± 0.09	0.82 ± 0.10	0.63 ± 0.10	0.0962
Beta-carotene (μg/mL)*	0.11 ± 0.01	0.13 ± 0.02	0.12 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.11 ± 0.01	0.3687
Lycopene (μg/mL)*	0.03 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.05 ± 0.03	0.3616

*For Fe analysis, N=11 for Ethanol, No Vit group and N=9 for Ethanol + Vit group

*For β-carotene, N=10 for Control, no Vit group, N=4 for Control + Vit group, N=9 for Ethanol, No Vit group, N=8 for Ethanol + Vit group, N=4 for Ethanol + Vit + choline group

*For Lycopene, N=7 for Control, no Vit group, N=4 for Control + Vit group, N=4 for Control + Vit group + choline group, N=9 for Ethanol, No Vit group, N=8 for Ethanol + Vit group, N=2 for Ethanol + Vit + choline group

Table 4: Analysis by Ethanol and Vitamin

	Control, no Vit	Control + Vit	Control + Vit + choline	Ethanol, No Vit	Ethanol + Vit	Ethanol + Vit + choline	P value Etoh	P value Vitamin	P value Etoh X Vit
N	11	6	4	12	10	6			
TRAP (μM Trolox Equivalents)	173.9 ± 10.5	174.3 ± 13.0	157.8 ± 13.0	163.6 ± 12.6	157.3 ± 7.3	147.3 ± 11.2	0.2343	0.4553	0.9527
TBARs (μM MDA) *	1.10 ± 0.10	1.28 ± 0.13	1.07 ± 0.07	1.07 ± 0.11	1.26 ± 0.07	0.77 ± 0.19	0.2502	**0.0498**	0.5211
Cp activity (Units/L)	229.3 ± 15.4	253.1 ± 16.4	234.2 ± 23.3	219.4 ± 12.2	214.4 ± 13.8	185.0 ± 21.6	**0.0269**	0.4531	0.4595
Zn (μg/mL)	0.64 ± 0.02	0.63 ± 0.01	0.68 ± 0.05	0.57 ± 0.03	0.54 ± 0.02	0.62 ± 0.04	**0.0038**	0.1370	0.8417
Cu (μg/mL)	1.89 ± 0.08	2.01 ± 0.08	1.74 ± 0.16	1.79 ± 0.08	1.81 ± 0.07	1.51 ± 0.13	**0.0296**	**0.0363**	0.7690
Fe (μg/mL) *	0.78 ± 0.10	0.87 ± 0.06	0.85 ± 0.08	0.73 ± 0.10	0.72 ±0.10	0.94 ± 0.16	0.7173	0.4787	0.6410
Mg (μg/mL)	15.94 ± 0.23	16.92 ± 0.45	15.89 ± 0.41	15.80 ± 0.22	15.85 ± 0.32	15.97 ± 0.44	0.1864	0.2151	0.2143
Ca (μg/mL)	82.29 ± 0.82	85.54 ± 1.22	83.94 ± 1.30	84.10 ± 1.97	83.65 ± 2.09	81.49 ± 1.53	0.5936	0.6090	0.4198
Vitamin A retinol (μg/mL)	0.70 ± 0.07	0.67 ± 0.05	0.68 ± 0.07	0.79 ± 0.07	0.66 ± 0.06	0.82 ± 0.08	0.2322	0.4229	0.6263
Vitamin E α-tocopherol (μg/mL)	27.22 ± 1.57	29.77 ± 1.63	34.46 ± 2.14	31.19 ± 2.01	33.43 ± 2.12	27.90 ± 4.15	0.8617	0.5041	0.1018
Choline (μM)	7.22 ± 0.52	7.94 ± 0.66	6.03 ± 0.60	8.53 ± 0.47	7.29 ± 0.45	8.28 ± 0.40	**0.0449**	0.4743	0.0550
Dimethyl glycine (μM)	1.40 ± 0.16	1.55 ± 0.11	1.24 ± 0.12	1.30 ± 0.21	1.53 ± 0.12	1.67 ± 0.31	0.5480	0.5991	0.4591
Betaine (μM)	12.45 ± 1.18	12.52 ± 1.39	11.68 ± 1.63	12.85 ± 0.53	11.71 ± 1.10	14.63 ± 1.35	0.3967	0.7298	0.3707
Lutein (μg/mL)	0.72 ± 0.08	1.05 ± 0.12	0.62 ± 0.07	0.75 ± 0.09	0.82 ± 0.10	0.63 ± 0.10	0.4690	**0.0189**	0.3272
Beta-carotene (μg/mL)*	0.11 ± 0.01	0.13 ± 0.02	0.12 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.11 ± 0.01	0.9210	0.4705	0.2067
Lycopene (μg/mL)*	0.03 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.05 ± 0.03	0.6373	0.1749	0.3910

*For TBARs analysis, N=5 for Control + Vit group

*For Fe analysis, N=11 for Ethanol, No Vit group and N=9 for Ethanol + Vit group

*For β-carotene, N=10 for Control, no Vit group, N=4 for Control + Vit group, N=9 for Ethanol, No Vit group, N=8 for Ethanol + Vit group, N=4 for Ethanol + Vit + choline group

*For Lycopene, N=7 for Control, no Vit group, N=4 for Control + Vit group, N=4 for Control + Vit group + choline group, N=9 for Ethanol, No Vit group, N=8 for Ethanol + Vit group, N=2 for Ethanol + Vit + choline group

Table 5: Analysis by Vitamin Supplement

	No Vitamin	Plus Vitamin	Plus Vitamin Plus Choline	P value
N	23	16	10	
TRAP (µM Trolox Equivalents)	168.6 ± 8.2	163.7 ± 6.8	151.5 ± 8.2	0.4058
TBARs (µM MDA)	1.09 ± 0.07[ab]	1.27 ± 0.06[a]	0.89 ± 0.12[b]	**0.0244**
Cp activity (Units/L)	224.1 ± 9.6	229.0 ± 11.3	204.7 ± 17.1	0.4291
Zn (µg/mL)	0.60 ± 0.02	0.57 ± 0.02	0.64 ± 0.03	0.1272
Cu (µg/mL)	1.84 ± 0.06[a]	1.88 ± 0.06[a]	1.60 ± 0.10[b]	**0.0316**
Fe (µg/mL) *	0.75 ±0.07	0.78 ± 0.07	0.90 ± 0.10	0.4011
Mg (µg/mL)	15.87 ± 0.16	16.26 ± 0.29	15.94 ± 0.30	0.4275
Ca (µg/mL)	83.23 ± 1.09	84.36 ± 1.37	82.47 ± 1.08	0.6257
Vitamin A retinol (µg/mL)	0.74 ± 0.05	0.66 ± 0.04	0.76 ± 0.06	0.3366
Vitamin E α-tocopherol (µg/mL)	29.29 ± 1.33	32.06 ± 1.49	30.52 ± 2.74	0.4592
Choline (µM)	7.90 ± 0.37	7.53 ± 0.37	7.38 ± 0.49	0.6450
Dimethylglycine (µM)	1.35 ± 0.13	1.53 ± 0.08	1.50 ± 0.19	0.5465
Betaine (µM)	12.66 ± 0.61	12.01 ± 0.84	13.45 ± 1.09	0.5371
Lutein (µg/mL)	0.73 ± 0.06[ab]	0.91 ± 0.08[a]	0.62 ± 0.06[b]	**0.0330**
Beta-carotene (µg/mL)*	0.12 ± 0.00	0.13 ± 0.01	0.12 ± 0.01	0.4458
Lycopene (µg/mL)*	0.03 ± 0.00	0.04 ± 0.01	0.05 ± 0.01	0.1680

*For TBARs analysis, N=15 for Plus Vitamin group
*For Fe analysis, N=22 for No Vitamin group and N=15 for Plus Vitamin group
*For β-carotene, N=19 for No Vitamin group, N=12 for Plus Vitamin group, N=8 for Plus Vitamin Plus Choline group
*For Lycopene, N=16 for No Vitamin group, N=12 for Plus Vitamin group, N=6 for Plus Vitamin Plus Choline group

Table 6: Analysis by Trimester

	N	TRAP (μM Trolox Equivalents)	TBARs (μM MDA)	Cp activity (Units/L)	Zn (μg/mL)	Cu (μg/mL)	Fe (μg/mL) *	Mg (μg/mL)	Ca (μg/mL)
1st Tri									
Control, No Vit	2	169.1 ± 2.1	1.24 ± 0.11	182.3 ± 1.5	0.65 ± 0.07	1.83 ± 0.09	0.89 ± 0.37	15.78 ± 0.19	84.67 ± 1.25
Control + Vit	1	186.2	0.94	216.9	0.66	1.714	0.67	17.78	89.76
Control + Vit +choline	1	186.7	0.87	200.4	0.75	1.53	0.85	15.55	83.91
Ethanol, No Vit	0								
Ethanol + Vit	1	172.3	1.22	191.3	0.51	1.62	0.83	15.14	80.32
Ethanol + Vit +choline	1	165.9	0.23	148.7	0.57	1.27	1.13	16.49	84.58
P value		0.2132	0.2575	0.0628	0.6448	0.3605	0.9665	0.1869	0.3713
2nd Tri									
Control, No Vit	8	174.0 ± 14.7	1.02 ± 0.13	235.3 ± 18.6	0.64 ± 0.03	1.89 ± 0.11	0.79 ± 0.10	16.01 ± 0.32	82.34 ± 0.76
Control + Vit	4	172.3 ± 20.2	1.37 ± 0.12	258.6 ± 23.1	0.63 ± 0.01	2.05 ± 0.08	0.93 ± 0.07	16.32 ± 0.34	85.45 ± 1.02
Control + Vit +choline	3	148.1 ± 12.2	1.14 ± 0.02	245.5 ± 28.8	0.66 ± 0.06	1.81 ± 0.21	0.85 ± 0.11	16.01 ± 0.56	83.95 ± 1.84
Ethanol, No Vit	10	156.6 ± 13.2	1.08 ± 0.13	215.9 ± 12.9	0.58 ± 0.03	1.76 ± 0.08	0.76 ± 0.11	15.86 ± 0.26	83.90 ± 2.38
Ethanol + Vit	5	160.7 ± 12.8	1.21 ± 0.12	215.1 ± 26.5	0.57 ± 0.02	1.81 ± 0.13	0.75 ± 0.17	16.48 ± 0.39	83.35 ± 1.15
Ethanol + Vit +choline	4	153.0 ± 11.5	0.82 ± 0.23	203.2 ± 28.9	0.67 ± 0.02	1.62 ± 0.17	1.07 ± 0.11	16.32 ± 0.35	82.27 ± 1.30
P value		0.8419	0.3545	0.5705	0.2208	0.3518	0.5542	0.7723	0.9036
3rd Tri									
Control, No Vit	1	183.4	1.492	274.9	0.59	2.04	0.42	15.71	77.17
Control + Vit	1	170.3		267.8	0.61	2.14	0.80	18.47	81.72
Control + Vit +choline	0								
Ethanol, No Vit	2	198.7 ± 34.3	1.06 ± 0.01	236.4 ± 45.1	0.52 ± 0.05	1.93 ± 0.38	0.58 ± 0.13	15.46 ± 0.23	85.11 ± 0.92
Ethanol + Vit	4	149.4 ± 9.3	1.32 ± 0.10	219.4 ± 14.2	0.50 ± 0.02	1.85 ± 0.04	0.64 ± 0.10	15.26 ± 0.44	84.86 ± 5.39
Ethanol + Vit +choline	1	106.1	1.11	148.5	0.44	1.29	0.23	14.08	75.30
P value		0.2506		0.3250	0.2659	0.3610	0.3377	0.0863	0.8443

*For TBARs analysis, N=0 for Control Plus Vitamin group. Too many missing groups for ANOVA (Third Trimester).
*For Fe analysis, N=9 for Ethanol, No Vitamin group (Third Trimester)

Table 6: Analysis by Trimester continued

	N	Vitamin A retinol (µg/mL)	Vitamin E α-tocopherol (µg/mL)	Choline (µM)	Dimethyl glycine (µM)	Betaine (µM)	Lutein (µg/mL)	Beta-carotene (µg/mL)*	Lycopene (µg/mL)*
1st Tri									
Control, No Vit	2	0.64 ± 0.03	27.20 ± 7.6	6.03 ± 0.80	1.67 ± 0.56	12.13 ± 0.47	0.70 ± 0.39	0.1 ± 0.01	
Control + Vit	1	0.74	26.68	6.00	1.41	9.00	1.16	0.16	
Control + Vit + choline	1	0.52	35.27	6.33	1.21	15.71	0.47	0.14	
Ethanol, No Vit	0		26.72						
Ethanol + Vit	1	0.92	23.41	6.81	1.9	12.39	0.76	0.18	
Ethanol + Vit + choline	1	0.92		7.34	0.96	18.18	0.37	0.10	
P value		0.1728	0.9275	0.8602	0.8905	0.1363	0.8493	0.2624	
2nd Tri									
Control, No Vit	8	0.75 ± 0.08	27.11 ± 1.66	7.69 ± 0.63	1.40 ± 0.18	12.92 ± 1.58	0.68 ± 0.06	0.11 ± 0.01	0.03 ± 0.01
Control + Vit	4	0.68 ± 0.07	29.70 ± 2.21	8.26 ± 0.84	1.52 ± 0.15	13.75 ± 1.78	1.05 ± 0.18	0.10 ± 0.01	0.04 ± 0.01
Control + Vit +choline	3	0.73 ± 0.07	34.19 ± 3.00	5.93 ± 0.83	1.25 ± 0.16	10.33 ± 1.29	0.67 ± 0.07	0.12 ± 0.02	0.06 ± 0.02
Ethanol, No Vit	10	0.83 ± 0.06	29.79 ± 1.98	8.44 ± 0.57	1.27 ± 0.25	13.01 ± 0.61	0.69 ± 0.07	0.13 ± 0.01	0.04 ± 0.01
Ethanol + Vit	5	0.67 ± 0.07	35.20 ± 3.69	7.21 ± 0.54	1.47 ± 0.18	13.43 ± 1.78	0.91 ± 0.17	0.13 ± 0.02	0.03 ± 0.01
Ethanol + Vit + choline	4	0.84 ± 0.10	24.00 ± 1.62	8.33 ± 0.52	2.03 ± 0.31	14.89 ± 1.30	0.60 ± 0.09	0.10 ± 0.01	0.02
P value		0.5335	0.0622	0.2495	0.4063	0.6243	0.1008	0.2506	0.5665
3rd Tri									
Control, No Vit	1	0.37	28.17	5.88	0.87	9.39	1.04	0.12	0.02
Control + Vit	1	0.54	33.17	8.62	1.78	11.12	0.95	0.15	0.05
Control + Vit +choline	0								
Ethanol, No Vit	2	0.56 ± 0.21	38.21 ± 5.54	8.97 ± 0.13	1.45 ± 0.02	12.08 ± 1.14	1.05 ± 0.35	0.12 ± 0.00	0.03 ± 0.00
Ethanol + Vit	4	0.58 ± 0.10	32.90 ± 2.41	7.51 ± 1.00	1.51 ± 0.19	9.38 ± 0.81	0.72 ± 0.14	0.11 ± 0.01	0.04 ± 0.02
Ethanol + Vit +choline	1	0.62	47.97	9.03	0.91	10.01	0.97	0.14	0.08
P value		0.9242	0.2660	0.6154	0.3000	0.4717	0.7906	0.0900	0.5432

*For β-carotene analysis (Second Trimester), N=7 for Control, No Vitamin group, N=2 for Control + Vitamin group, N=7 for Ethanol, No Vit group, N=4 for Ethanol + Vit group, N=2 for Ethanol +Vit +choline group

*For β-carotene analysis (Third Trimester), N=3 for Ethanol + Vit group

*For Lycopene analysis, Too many missing groups for ANOVA (First Trimester).

*For Lycopene analysis (Second Trimester), N=5 for Control, No Vitamin group, N=2 for Control + Vitamin group, N=7 for Ethanol, No Vit group, N=4 for Ethanol + Vit group, N=1 for Ethanol +Vit +choline group

*For Lycopene analysis (Third Trimester), N=3 for Ethanol + Vit group

Regression correlation 1: Influence of Weeks of Gestation

	TRAP (μM Trolox Equivalents)	TBARs (μM MDA)	Cp activity (Units/L)	Zn (μg/mL)	Cu (μg/mL)	Fe (μg/mL) *	Mg (μg/mL)	Ca (μg/mL)
N	49	48	49	49	49	47	49	49
Vs. Wks of gestation								
P value	0.7253	0.0188	0.1760	0.0001	0.0527	0.0002	0.0272	0.2146
R-squared		0.114		0.269	0.078	0.273	0.100	
Correlation		Positive		Negative	Positive	Negative	Negative	

*As weeks of gestation increase, Plasma Zn, Fe and Mg decrease and Plasma Cu and TBARs increase.

Regression correlation 2: Influence of Weeks of Gestation

	Vitamin A retinol (μg/mL)	Vitamin E α-tocopherol (μg/mL)	Choline (μM)	Dimethyl glycine (μM)	Betaine (μM)	Lutein (μg/mL)	Beta-carotene (μg/mL)*	Lycopene (μg/mL)*
N	49	49	49	49	49	49	39	34
Vs. Wks of gestation								
P value	0.0454	0.0012	0.0969	0.4786	0.0007	0.0103	0.4299	0.2288
R-squared	0.082	0.203			0.218	0.132		
Correlation	Negative	Positive			Negative	Positive		

*As weeks of gestation increase, Plasma Vitamin E and Lutein increase, and Plasma Vitamin A and Dimethylglycine decrease.

Problems:

The initially planned recruitment timeline for this study was for continued enrollment through year 3, with all pregnancy outcomes collected by Year 4, and all children receiving one year neurodevelopmental assessments before the end of Year 5. In Ukraine, we have exceeded planned recruitment rates by more than 50% and will complete the planned sample size recruitment a year ahead of schedule, with the potential to double the sample size recruitment in that location (n = 360) by mid-way through Year 3. However, in Russia, we have had multiple difficulties in proceeding with the protocol as planned. These include the inability to import the required chemicals for completing choline analyses, and changes in the government tax regulations regarding payment of personnel. A re-evaluation with collaborators of the sample recruitment strategy is underway in order to remain within the study timeline and to achieve the targeted enrollment and the ~400 infants required for sufficient power to detect the predicted neurobehavioral effect size.

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

This clinical project relates to nutritional aims of other projects in the Consortium. Choline measures at baseline in the human maternal samples have been compared to levels in tissue in the rat model being collected as part of Jennifer Thomas' basic science developmental project. In keeping with the aims of Dr. Goodlett's working group on biomarkers, a maternal hair sample collection protocol has been introduced in our clinical project in order to provide an additional biomarker of exposure. Similarly, in keeping with the aims of Dr. Foroud's working group on collection of DNA for study subjects, blood samples are being banked in our project to allow for genetic susceptibility analyses at a future time point. Our clinical project relies on the Dysmorphology Core and the Informatics Core for validation of diagnosis and for services of the data repository. Our clinical project relates to the developmental project of Dr. Hull, and has provided the 80 new subjects and resources for collection of the expanded ultrasound data. Our clinical project also relates to the neurobehavioral phenotype clinical project led by Dr. Mattson by providing infant neurobehavioral outcomes to help delineate the spectrum of phenotypes across the age range. These data will be available beginning in March of 2009. Finally, this project has offered subjects and resources for Dr. Foroud's 3D imaging clinical study should they choose to perform imaging on children between six and twelve months of age. Interaction between the Consortium investigators across projects has taken place on all of these levels. An abstract has

been presented in collaboration with Dr. Hull and Dr. Sulik comparing the mouse model to human ultrasound findings, and a manuscript is in preparation involving the nutritional hypotheses and preliminary findings in our clinical project to the basic science developmental project being conducted by Dr. Thomas.

VIII. Plans for the Next Six Months:

1. Complete folate and homocysteine analyses for blood samples.
2. Conduct analyses of 3rd trimester blood samples for repeated measures for micronutrients relative to treatment group assignment.
3. Complete the enrollment for planned sample in Ukraine.
4. Submit the two manuscripts now in preparation.
5. Submit abstracts to RSA and Teratology Society meetings on results of nutritional analyses, and outcomes with respect to physical features of FAS for the cumulative 210 pregnancies now in the study.
6. Continue validation of physical examinations of newborns by dysmorphology core March, 2009 and May, 2009.
7. Begin neurobehavioral testing in March/April, 2009.
8. Plan to begin 3D photography on six-month old infants March/April, 2009

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 Since Last Progress Report:

Sulik, KK, Moore SO'L, Parnell SE, Myers EA, Dehart DB, Johnson GA, Chambers CD, and Hull AD. High resolution magnetic resonance imaging of alcohol-exposed fetal mice confirms and informs human prenatal ultrasound studies. Abstract presented for DW Smith Workshop, August, 2008.

Chambers CD, et al. Predictors of binge drinking during pregnancy among women in Ukraine. Abstract poster presented at American Public Health Association meeting, October, 2008.

Chambers CD et al. Invited symposium speaker on CIFASD studies in Eastern Europe at First Central and Eastern European Summit on Preconception Health and Prevention of Birth Defects, Budapest, Hungary, August, 2008.

Sosynyuk, Z et al. Prenatal ultrasound to detect effects of in utero alcohol exposure. Abstract poster presented at First Central and Eastern European Summit on Preconception Health and Prevention of Birth Defects, Budapest, Hungary, August, 2008.

I. Principal Investigator and Key Personnel: Tatiana Foroud, Ph.D. – Principal Investigator, Elizabeth Moore, Ph.D. – Co-Investigator, Richard Ward, Ph.D. – Co-Investigator, Shiaofen Fang, Ph.D. – Co-Investigator, Li Shen, Ph.D. – Co-Investigator, Christian Klingenberg, Ph.D. - Consultant

II. Title of Project: Facial Imaging Project U01 AA014809

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.
The core has been expanded to also include the collection of saliva samples for the extraction of DNA. Saliva is being collected from the study participants and will be used in future analyses to identify genetic risk factors affecting fetal susceptibility to fetal alcohol syndrome.

IV. Methods:

The project is currently continuing to analyze data collected over the past 5 years and is now collecting data using a new 3D imaging system. The new system is operational in San Diego and Atlanta. Images will be collected by Facial Imaging Core staff at the Los Angeles site in January 2009. A pilot study was completed that imaged the same individuals using both systems. The images with the new 3dMD system are clearly of higher resolution and better quality. However, there is significant correlation between the measurements taken from the two cameras.

We have also completed all IRB documents and obtained approval to initiate the collection of saliva at two sites. We are currently working on the same documents to obtain saliva at a third site. The saliva will be used to extract DNA, which will be stored at Indiana University for future studies.

V. Accomplishments and Results:

Specific Aim 1: Improve understanding of the dysmorphic features in FAS.

The focus of this aim is the collection of images in the field from individuals with delineated prenatal alcohol exposure who have also undergone a dysmorphology evaluation and in most cases cognitive testing as well. Training has been completed at two sites and data collection has now begun.

We completed an intercamera study which imaged over 20 people using both our previous camera, the Minolta Vivid910fw and the 3dMD camera which is now used in our ongoing studies. The primary purpose of this intercamera study was to compare images collected from the two cameras to allow us to determine how best to combine data from the first 4 years of the study with the data that will be collected as part of the new study. The secondary purpose of this comparison was to develop a protocol that could be used at the sites for image collection.

We have also completed extensive comparisons of the diagnosis obtained directly by the dysmorphologist examining the subject in person with the diagnosis obtained by the dysmorphologist when viewing the 3D image. We have worked with 2 members of the Dysmorphology Core who have reviewed a common set of 125 images and provided a diagnosis. We have identified systematic differences between the dymorphologists in the classification of subjects using the 3D facial images.

Specific Aim 2: Enhance the capability of definitive diagnosis of fetal alcohol syndrome (FAS) and the broader spectrum of fetal alcohol spectrum disorders (FASD) at different stages of the lifespan.

As part of this aim, we have completed analyses from the previous study that has resulted in one published manuscript (Fang et al, 2008) and another manuscript that is currently under review (McLaughlin et al, submitted). Both manuscripts have focused on novel feature extraction and in the former compared subjects from South African and Finland and the latter analyses compared only subjects from South Africa. These analyses allowed us to test and compare new methods in subjects who were relatively young (between 3-10 years; South African) and across a wider age range, but primarly older adolescents (Finland).

Our consultant, Dr. Christian Klingenberg has initiated analyses to assess asymetry in the FAS cases and controls. Using data from the original camera and from the 4 study groups previously analyzed in Moore et al (2007) he has found significant evidence of fluctuating asymetry in the FAS group. Results are currently being prepared for publication. Additional analyses are currently underway with Dr. Shen, a new study investigator, who has been developing quantitative measures of the philtrum surface and the upper lip volumes.

Specific Aim 3: Establish whether there is a relationship between FAS dysmorphic features and the specific underlying impairments in brain function.

The clinical aspects of this aim have not yet been initiated since there are only a limited number of subjects who have completed both 3D facial imaging and MRI. However, we anticipate that in the coming year a growing number of subjects will be participate in both protocols and will provide a cohort that can be studied more intensively.

Ongoing interactions have been fostered between the investigators utilizing animal models and the members of this project. A monthly meeting is held by both this project and the project of Feng Zhou utilizing mouse models. Investigators from both projects attend both meetings, leading to a robust interaction between the two groups. There have also been regular meetings with Dr. Charles Goodlett who is a part of the sheep project of Dr. Cudd. Dr. Elizabeth Moore has also worked directly with Drs. Goodlett and Cudd in training the site investigators in the collection of anthropometric data from the sheep.

Specific Aim 4: Collection of DNA from study participants

We have initiated the collection of saliva from subjects participating in the San Diego and Los Angeles sites and are currently working on the IRB protocols for the Atlanta site. The saliva will be shipped to Indiana University where it will be extracted.

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. Collection of DNA is a new priority of the core.

VII. Interrelation with the 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 collecting larger numbers of images with the new 3dMD system at the study sites. We will also be collecting saliva and blood samples for DNA extraction.

IX. Publications:

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. 11(3):162-71, 2008 Aug;

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:

None

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 U01 AA014834

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 symptomology. 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, 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 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 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:

Funding for Phase II began on September 30, 2007, significant progress has been achieved as follows:

A. General Progress (San Diego):

1. *IRB Approvals*. We have active IRB approval for the neuropsychological, dysmorphology, brain imaging, and 3D imaging portions of the study at each site (where relevant).
2. *Subcontracts*. All three subcontracts are active.
3. *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 South Africa which is waiting for funds to be released to hire personnel.
4. *Purchasing*. Necessary materials and equipment are in place.
5. *Material Development*. We have finalized versions of test administration and scoring materials.
6. *Training*. We conducted two training meetings in San Diego on December 3-5, 2007 (data collection staff from University of New Mexico and UCLA) and January 14-15, 2008 (all sites present). Participants were trained on data collection procedures, scoring, and received materials for pilot testing and instructions for reliability procedures. 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. We have received and approved pilot materials from UCLA (2 testers), UNM (1 tester). We receive pilot materials from Atlanta (1 tester) on 11/5/2008 and provided feedback on 11/19/2008. We are waiting to receive materials from a second pilot subject. We have not received pilot materials from South Africa.
7. *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 it is currently pending deployment, as far as we know. We are also working with them on development of the neuro demographics database, which has also gone through several iterations and is getting closes to a final version.

B. Neurobehavioral Testing: Data collection for Phase II has begun at most sites, and a total of 32 subjects have been tested, see below for details. In San Diego, we have tested 13 subjects using the Phase II test battery. We are also continuing to collect data from a small number of subjects using the Phase I test battery. To date we have tested 114 children using this test battery and plan to test 2 more. This sample includes 52 FASD, 40 CON, and 18 or 19 children with ADHD (one child may actually be moved to the FASD group).

C. MRI Evaluations: The San Diego site is also involved in the brain imaging project (E. Sowell, PI). We have IRB approval for this component, have piloted the procedures and are currently screening subjects for eligibility. Of the 13 children tested using the neurobehavioral test battery, 1 has been scheduled for the mock scanning session and will be scheduled for scanning upon successful completion of that session. Of the remaining children, 6 are ineligible, 6 have not yet been screened. One additional child who is scheduled for neurohavioral testing has been screened and scheduled for the mock session also. Mock scanning sessions are scheduled for 12/8/2008 and

12/9/2008. We also collected MRI data from 43 subjects at the San Diego site during the CIFASD Phase I funding period.

D. 3-D Facial Imaging: In San Diego, we received the 3-D camera at the beginning of April 2005 with the original camera. We received the new camera in July of 2008. Since that time, we have evaluated 117 children and transferred these data to Elizabeth Moore.

E. Dysmorphology: Since the beginning of the CIFASD Phase I, 115 children have been examined by the dysmorphology core at the San Diego site.

F. Genetics: We have obtained IRB approval to collect saliva for genetic testing under the direction of Dr. Tatiana Foroud. Standardized procedures are being developed and materials distributed. No data have been collected.

G. Site-Specific Progress: All sites have approved IRB protocols and are awaiting approval. All sites attended one or both training meetings in December 2007 and January 2008. The next training meeting is being planned for early 2009. Our budget allowed data collection to begin in month 7 (April 2008). Data collection began at the sites as follows:

1. San Diego: Data collection began June 2008 and thus far, 13 subjects have been tested using the Phase II neurobehavioral test battery. Six subjects are currently scheduled for testing. Dysmorphology and 3D photography are in progress and we are screening subjects for brain imaging. We have IRB approval to collect saliva for the genetic study being conducted by Dr. Foroud.
2. UCLA: Data collection began in May of 2008 and thus far, 14 subjects have been tested using the Phase II neurobehavioral test battery. Six subjects are currently scheduled for neurobehavioral testing. Dysmorphology exams are scheduled for January 15-16, 2009 and 3D photography is expected to be conducted at that time.
3. Atlanta: Data collection has not begun. The site is in the process of training and pilot testing. A second pilot is in progress and will be reviewed by the SDSU site when available. Dysmorphology exams are scheduled for December 5-6, 2008 and 3D photography is expected to be conducted at that time.
4. UNM/Plains: Data collection was approved to begin in April, 2008 and thus far, 2 subjects have been tested using the Phase II neurobehavioral test battery.
5. UNM/UNM: Data collection was approved to begin in April, 2008 and thus far, 3 subjects have been tested using the Phase II neurobehavioral test battery.
6. UNM/South Africa: Data is not being collected yet.

Obstacles and Plans for Overcoming Obstacles:

Our biggest obstacle is in getting data collection to commence and continue in a reliable and valid fashion. Two sites are reliable and collecting data at a regular rate (SDSU, UCLA). Two sites (UNM, Plains) are reliable and collecting data but at a slower rate. The next trip to the Plains is currently being planned. Having a second tester at this site would be helpful and is budgeted. The UNM site is taking steps to recruit subjects (See Appendix 1, below). The third site in the UNM subcontract, UCT, is behind schedule. The subcontract was only just finalized and funds are not yet available. This has delayed all project activities. On a recent trip to SA, the PI and site PI discussed plans for 2009 and hiring is in progress. Training of the two testers will be challenging. We anticipate a training meeting in San Diego in early 2009, which hopefully will involve either the site PI or at least one of the testers. One site (Marcus) is not yet reliable and is

therefore not collecting data. Training was delayed due to the administrative changes at the site (see Appendix 2, below). Pilot testing was reviewed by the main site and a second pilot was required. This pilot is in progress. Once reliable, data collection should commence and proceed in a timely fashion.

A second area of weakness is data on alcohol exposure for Phase I subjects. We have been working on the "demographics" database with the informatics group and it is near completion. This database will provide information on alcohol exposure for all Phase II subjects but will require sites who participated in Phase I to go back and enter data. No new data collection is required but files for each subject will need to be reviewed. The data sheet necessary to collect these data has been distributed to all sites in Phase II, but it is unlikely that some of the Phase I sites (Finland, Moscow) will participate in this activity.

A more minor area of weakness is timing of the 3D photographs. We have routinely been taking them when children are tested with the neurobehavioral test battery. However, we have had some children seen by Dr. Jones for dysmorphology and have not had their photographs taken because we were unclear about whether they would qualify for the study. These are subjects for which exposure data were not clear and we were unsure they would meet our criteria for inclusion. If enrolled in the neurobehavioral aspect of the study, they will have a photograph taken. But there may be some children with dysmorphology and no 3D photography (or neuropsych testing).

Other than data collection, all other aspects of the project are in place. We have designed or purchased and deployed all the training and administration materials, we have been working with the informatics core on the databases and these are near completion. Once data collection begins at all sites, it is likely to proceed quickly.

VI. Discussion: In the 14 months since funding began, we have made considerable progress at some sites. Challenges remain at other sites and data collection has not been as active as we had hoped at these sites. Ultimately, data collection will be conducted at multiple sites with varied subject characteristics which will put us in the position to have access to a large, heterogeneous population on which to test our hypotheses.

VII. Interrelation to the 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 have reliable and valid data collection ongoing at all 6 sites using 3D photo, dysmorphology, brain imaging, and neurobehavioral testing. We will continue to provide administrative support as well as ongoing training and reliability assessment 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 from the last year (1/2008-12/2008):

Fang, S., McLaughlin, J., Fang, J., Huang, J., Autti-Rämö, I., Fagerlund, Å., Jacobson, S.W., Robinson, L.K., Hoyme, H.E., Mattson, S.N., Riley, E., Zhou, F., Ward, R., Moore, E.S., Foroud, T., and the CIFASD (2008). Automated diagnosis of fetal alcohol syndrome using 3D facial image analysis. Orthodontics and Craniofacial Research, 11, 162-171. doi: 10.1111/j.1601-6343.2008.00425.x http://www3.interscience.wiley.com/journal/120755909/abstract

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

Aragón, A.S., Coriale, G., Fiorentino, D., Kalberg, W.O., Buckley, D., Gossage, J.P., Ceccanti, M., Mitchell, E.R., and May, P.A. (2008). Neuropsychological Characteristics of Italian Children with Fetal Alcohol Spectrum Disorders. Alcoholism: Clinical and Experimental Research, 32 (11),1909-1919.

Aragón, A.S., Kalberg, W.O., Buckley, D., Tabachnick, B.G., Barela, L.M., and May, P.A. (2008). Neuropsychological Study of FASD in a Sample of American Indian Children: processing simple versus complex information. Alcoholism: Clinical and Experimental Research, 32 (12), 2136-2148.

Fryer, S.L., Schweinsburg, B.C., Bjorkquist, O.A., Frank, L.R., Mattson, S.N., Spadoni, A.D, and Riley, E.P. (In Press 2008). Characterization of white matter microstructure in fetal alcohol spectrum disorders. Alcoholism: Clinical and Experimental Research.

Papers under review:

Fagerlund, Å., Korkman, M., Autti- Rämö, I, Mattson, S.N., and Hoyme, H.E. (Submitted 2008). Risk and protective factors for mental and behavioral well-being in fetal alcohol spectrum disorders.

Spadoni, A.D., Bazinet, A.D., Fryer, S.L., Tapert, S.F., Mattson, S.N., and Riley, E.P. (Submitted 2008). BOLD response during spatial working memory in youth with heavy prenatal alcohol exposure.

Bjorkquist, O.A., Fryer, S.L., Reiss, A.L., Mattson, S.N., Riley, E.P. (Submitted 2008). Cingulate gyrus morphology in children and adolescents with fetal alcohol spectrum disorders.

Lu, L.H., Houston, S.M., Nuñez, C., O'Hare, E.D., Bookheimer, S.Y., Smith, L.M., O'Connor, M.J., Mattson, S.N., and Sowell, E.R. (Submitted 2008). Preliminary findings on neuropsychological effects of prenatal methamphetamine and alcohol exposure.

O'Hare, E.D., Lu, L.H., Houston, S.M., Bookheimer, S.Y., Mattson, S.N., O'Connor, M.J, and Sowell, E.R. (Submitted 2008). Altered frontal-parietal functioning during verbal working memory in children with heavy prenatal alcohol exposure.

Papers in preparation:

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.

Mattson, S.N., Roesch, S.C., Riley, E.P., et al. Neurobehavioral Profile of Children with heavy prenatal alcohol exposure

Mattson, S.N., et al. The Collaborative Initiative on Fetal Alcohol Spectrum Disorders: Methodology for Clinical Projects

X. Posters and presentations (1/2008-12/2008):

Vaurio, L., Kang, N., Wagner, A., Riley, E.P., and Mattson, S.N. (2008). Laboratory validation of parent-reported measures of inattention and hyperactivity in children with heavy prenatal alcohol exposure. Presented at the Research Society on Alcoholism meeting, Washington, DC, June 2008. Alcoholism: Clinical and Experimental Research, 32 (6), 135A.

Kang, N., Vaurio, L., Doughty, R.S., Riley, E.P., and Mattson, S.N. (2008). Objective measurement of activity levels in children with heavy prenatal alcohol exposure. Presented at

the Research Society on Alcoholism meeting, Washington, DC, June 2008. Alcoholism: Clinical and Experimental Research, 32 (6), 136A.

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. Presented at the International Neuropsychological Society meeting, Waikaloa, February 2008. Journal of the International Neuropsychological Society, 14, Supplement S1. DOI: 10.1017/S1355617708080429, Published online by Cambridge University Press 12 Mar 2008, http://journals.cambridge.org/action/displayIssue?jid=INS&volumeId=14&issueId=S1

Appendix 1: Progress Report from Dr. Phil May, PI of UNM/Plains/SA Sites

CIFASD Neurobehavioral Project – Progress Report
November 25, 2008

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

Key Personnel: Alfredo Aragon, Ph.D.
David Buckley, M.S.
Wendy Kalberg, M.A., CED

We have completed the Institutional Review Board (IRB) approval process for all consents through the University of New Mexico as well as the Indian Health Services (IHS). We successfully gained approval for the work with each tribe through tribal resolutions.

Alfredo Aragon, Ph.D., attended training the week of January 14, 2008 in San Diego. Alfredo Aragon has successfully tested five children for the consortium; two children in Albuquerque, two children in Great Falls, Montana, and one child in Farmington, New Mexico. Arrangements are being made to have the New Mexico children seen by a dysmorphologist (either Gene Hoyme or Luther Robinson) the week of January 20, 2009. All evaluations are being video-taped according to the outlined protocol.

We are continuing to build a referral process in New Mexico for potential research clients. Furthermore, further planning and the exact timing of the data collection for the Plains sites will be undertaken next week as we hold our detailed scheduling meeting for the entire next calendar year of our various projects. The New Mexico site is behind schedule at this time, but steps are being taken to remedy this.

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

South African Site
Colleen Adnams, M.D., Co-PI
University of Cape Town, Department of Psychiatry

Dr. Adnams visited San Diego in January 2008 for training on the test battery. UCT IRB approval was received in February 2008. The subcontract for FY 1 was received from The University of New Mexico and finalised in September / October 2008. Access to spending of study funds is in process, which will facilitate recruitment of project staff.

Planning for 2009 was discussed in a site visit to South Africa made by Wendy Kalberg and Phil May in November. We have had some scheduling challenges caused by demands from other projects now underway. But we believe that these and also the division of labor and time allocation for four separate projects are now clear.

Current plans include the hiring of a study co-ordinator and two part-time test administrators. In Wellington a field worker has been hired and a test facility in the town of

Wellington (where most of the children – cases and controls live) has been equipped. It is located in the same building as the FASER-SA prevention research offices. We anticipate that translation into Afrikaans, and training of new staff on administration of the test battery will be completed in early 2009, after which testing will be piloted. Data collection will commence in approximately March 2009.

The South African site is behind schedule for FY1 and 2. However, given that the cohort of potential participants for the study is already identified from previous epidemiological studies, it will be possible to accelerate the number of subjects assessed during the coming year in order to catch up with data collection.

Because of the nine and ten hour time differential between Cape Town and New Mexico and San Diego, Dr. Adnams has not been expected to call in for the conference calls. Instead, the New Mexico staff members correspond and converse regularly with Dr. Adnams, and up to three visits a year are made to South Africa for coordinating all of our activities there, including this project.

Appendix 2: Progress Report from Dr. Claire Coles, PI of Atlanta Site

CIFASD Neurobehavioral Project – Progress Report
November 25, 2008

Subcontract: Claire Coles, Ph.D.
Marcus Institute, Emory University

Specific Aims: Unchanged
Studies and Results:

The Marcus Subcontract is collecting data in three areas:
1) Neurodevelopment; 2) Dysmorphia; 3) 3-Dimensional Imaging.

In support of these goals, we have carried out the following activities:

IRB approval
On receiving the notice that the contract had been approved in September, 2007, we initiated the application process to receive Human Subjects approval for this protocol from the Emory University Internal Review Board. The application was completed at the end of September 2007 and approved by Emory as of 12/31/07. The application for renewal of this approval, with modifications due to changes in contract administration and improved recruiting materials was submitted 11/24/08.

Transfer of Grant to Emory
Marcus Institute, where the FAS Clinic is located, was administered by Kennedy-Krieger Institute (KKI) located in Baltimore, MD. In the early part of 2008, KKI entered into negotiations with Children's Health Care of Atlanta (CHOA) to sell the building and other interests associated with Marcus. When this process began, KKI instructed that all grants and contracts should be transferred to other agencies. To assure the continuation of the contract, we initiated this process with the parties involved in the early part of 2008 and it was completed in June 2008 when Emory University School of Medicine assumed the contract.

Staff Hiring and Training
To carry out the activities of this contract, we required support in the following areas: Project Coordination, neuropsychological testing, subject recruitment. In April, with funds advanced by Emory University, we transferred an experienced Project Coordinator, Julie Carroll, LCSW, to this project. With this support we were able to begin pursuing project goals. Similarly, we provided an experienced recruiter to this project to identify clinic clients and controls who would be eligible for recruitment. Finally, in July, 2008, we recruited a graduate student, Tameka Jackson, in clinical psychology to be trained as a part time (one day per week) tester. Dr. Kable attended a workshop in December 2007 in San Diego to be trained in the protocol so that she would be able to supervise the student testers. An initial pilot was completed on 9/20. Pre-screening by the investigators determined that an additional pilot should be conducted to improve tester administration. Repeat sessions with pilots were completed on 10/18 and 10/25. The final pilot on 10/25 was submitted for review but was not approved. Currently, the tester is reviewing detailed feedback along with the video taped sessions in order to address the issues identified. A repeat pilot is scheduled for 12/9/08 and the video submission will be resubmitted for tester approval shortly afterwards. In addition, we are assigning a second tester, a post

doctoral fellow, to this activity to allow a higher volume of testing. Ms. Yang will begin training in December.

Access to 3-D camera and Training
To carry out the 3-D imaging component of this project, access to an appropriate camera is required. Dr. Coles identified such a camera, presently installed in the Emory Genetics Clinic, and negotiated access to it without cost. The camera will be available to the study on Fridays at their clinical site. An initial training session was provided by Jeff Rodgers from Indiana University on 6/6/08 with the principal investigators and the Project Coordinator who will be the primary camera technician. Staff were trained in using the 3D camera protocol and a preliminary training on facial landmarks. The Project Coordinator arranged for continued practice sessions on 7/30, 9/5, 9/12, 11/14 and 11/21 in order to develop her skills using the equipment. Practice sessions were often coordinated with Jeff Rogers to troubleshoot any problems with the computer and camera. Currently, several sets of practice images have been downloaded from the computer at the Genetic lab, and we are awaiting access to the Indiana file server in order to upload the images. A training session on uploading is scheduled for 12/1. We anticipate these images will be reviewed and feedback provided to the Project Coordinator prior to the upcoming camera imaging sessions scheduled on 12/5 and 12/6.

Subject Recruitment
To meet the requirements of the protocol, we will need to evaluate about 100 to 125 children ages 8 to 17. Of these up to 50 will be alcohol-exposed. These individuals are being recruited from the Marcus Clinic database and from the Clinic as patients are identified. In addition, members of three control populations are being recruited, normal controls, individuals whose IQ scores are between 55 and 88, and patients with ADHD. Although these cells will be more difficult to fill, we have developed recruiting materials and brochures to inform parents and professionals about the opportunities to participate in this research and these are currently being reviewed by the Emory IRB. By reviewing clinic files, a database of 159 subjects was created from our clinic population who were possible recruits based on age, FAS diagnosis, or alcohol exposure. For initial recruitment, approximately 30 families were called and 18 participants scheduled for examinations by Dr. Jones and 3D imaging on 12/5 and 12/6. A further 3 subjects were excluded from contrast groups because of suspected alcohol exposure. The next stage of recruitment will focus on identifying non-alcohol exposed groups for the contrast groups.
At present, we do not anticipate significant problems in subject recruitment and are delayed in data collection only by staff training issues.

Scheduling Data Collection in collaboration with Dysmorphology Core.
Working with Kenneth L. Jones, MD, Director of the Dysmorphology Core, we have scheduled a two-day session December 5th and 6th in Atlanta during which Dr. Jones will see approximately 18 research participants. We have arranged with Emory Genetics to allow this activity to be carried out in their Clinic to allow the 3-D imaging to be done at the same time.

Obstacles and Plans for Overcoming Obstacles
Delay of funding.
The subcontractor was denied funding until the IRB approval was completed. This provision prevented work on the contract except that done by the PI and the Co-PI, who provided in kind work on the project for a number of months to complete all the IRB and neuropsychological training activities. While this provision did not delay the IRB approval since the PI initiated this process as quickly as possible, it did prevent any other personnel from beginning work on the Project and prevented the hiring of staff who might otherwise have been working on

neuropsychological training and subject recruitment. Unfortunately, at the time IRB approval was finally received, other events intervened, ultimately requiring the transfer of the contract to a different agency (From the Marcus Institute to Emory University). In January, 2008, when we would normally have begun the hiring process to identify testers and recruiters, Marcus imposed a hiring freeze on all staff. This later proved to be the initial step in a sale of the Clinic to Children's Health Care of Atlanta. The PI was not aware of these plans when the contract was applied for. We do not anticipate further difficulties in this area.

Delays due to Acquisition of Marcus Clinic by Children's Health Care of Atlanta
Due to changes in the administration at Marcus (see above), we were not able to receive any funds until June, 2008. In April and May, we were able to hire and transfer some staff with the support of Emory University who advanced us funds to do so. Thus the combination of the delays resulting from the requirement that the IRB be approved and the change in Marcus ownership delayed subject recruitment significantly. We do not anticipate further problems in this area.

Lack of 3-D camera.
We did not have access to a 3-D camera but were able to obtain access to one at Emory Genetics. After consultation and a trip to Atlanta by Jeff Rogers, it was established that the camera was suitable for the purposes of the study. As mentioned above, there is no 3D camera available at our Marcus testing site. Therefore, we had to work with Human Genetics Department to arrange for possible clinical time to use their camera for our study. Due to their clinical schedule, Friday is the primary day available to our study for uninterrupted use of the camera imaging room. While we hoped to complete the imaging on the same day as neuropsychological testing, this was not possible due to the logistics of traveling to 2 separate locations for our participants. Therefore, we have determined to do multiple imaging sessions in conjunction with the physical exams by Ken Jones. Also, the Project Coordinator has worked out a protocol for transferring imaging data to Indiana University since the data will be stored off site. We do not anticipate further problems in this area.

Staff training
Presently, we are delayed in initiating data collection because our tester has not been found reliable by San Diego. We have provided Ms. Jackson with this feedback and provided more training. We have scheduled another pilot administration. We have also identified a second tester, at no cost to the project, and will try to bring her up to standard by the end of the year. It is regrettable that the initiation of the project was delayed by the IRB issue and the transfer of the grant because a previous well prepared tester had to leave during that period. As soon as the tester is approved, we can begin collection of this part of the data. We anticipate that this problem will be resolved shortly.

Data Collection
We have access to a number of alcohol-affected individuals. Once data collection is possible, we can move fairly quickly in recruitment and testing and 3-D imaging. Although we imagine that the control groups will be more of a challenge than the alcohol-affected groups, we anticipate that we can identify and test an adequate number of adolescents in all categories over the next 3 years to meet study goals.

Other Activities-Genetics study. We have decided to delay activities on this aspect of the project for two reasons. First, we have limited time and do not want to detract from the other areas of focus. Secondly, we have no funds to carry out any of these activities.

Significance: Unchanged
Plans

Publications: NA
Enrollment Table: Attached.

Appendix 3: Progress Report from Dr. Elizabeth Sowell, PI of UCLA Site

CIFASD Neurobehavioral Project – Progress Report
November 25, 2008

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

UCLA has made significant progress in meeting its project goals over the last year. Staff trained in administration of neuropsychological and parent measures at SDSU in December 2007 and January 2008, and the primary research assistant met reliability standards established by SDSU in May 2008. Subjects began participating in May 2008, and since then six exposed children (mean age=12.7, 6 males) and eight unexposed children (mean age=11.0, 3 males) have participated in the study, including completion of the neuropsychological battery and parent interviews/behavioral measures. In addition, six exposed children are currently scheduled to participate through February 2009. While no children diagnosed with ADHD have participated yet, active recruitment continues for participants in the ADHD group, and Dr. James McCracken, Director of Child Psychiatry at UCLA, is assisting with ongoing recruitment efforts. The subject total goal through December 2008 was 12-20 subjects, but delays in subcontract funding impeded progress. With subjects scheduled through February 2009 and continued recruitment efforts in place, it is expected that UCLA will be at or close to its February 1, 2009 goal of 19-24 subjects. Dr. Ken Jones is scheduled to conduct dysmorphology exams on alcohol exposed subjects on January 15 and 16, 2009, and it is expected that 3-D photographs will also be taken at that time. Given the expected increase in subjects, a new research assistant was hired in July 2008. Dr. Christopher Nuñez, post-doctoral fellow and clinical neuropsychologist, has dedicated significant effort to training the new research assistant on neuropsychological test administration and ensuring that she meets reliability standards. It is also expected that the addition of this staff member will be instrumental in increasing subjects totals. Finally, IRB approval has been obtained for collection of saliva samples, and we will begin collecting genetic data in December 2008.

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

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

III. Objectives:

In our proposal, we outlined 4 specific aims:
Specific Aim 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.

Specific Aim 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.

Specific Aim 3: To determine whether the anatomical "phenotype" relates to neurobehavioral profiles in children with fetal alcohol syndrome or FASDs.

Specific Aim 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:

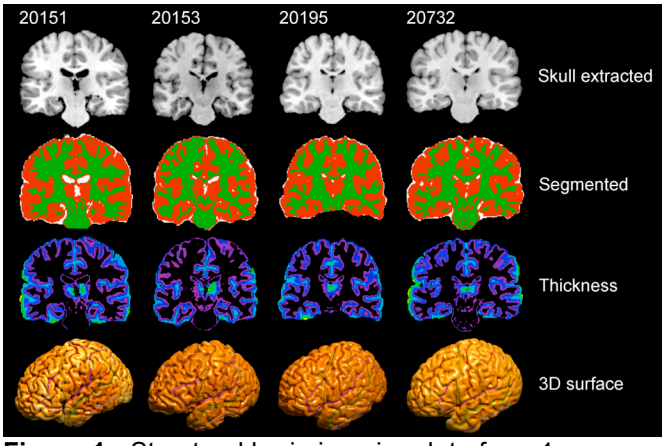


Figure 1: Structural brain imaging data from 4 new CIFASD participants.

Brain Imaging:
UCLA: Structural MRI data has been collected for 13 CIFASD subjects. Three to four hi-resolution MPRAGE scans were acquired per subject. Of the structural imaging data collected, 95% of the total scans were of acceptable quality. Only one subject was excluded from the analysis due to excessive movement. Preprocessing steps have been completed on all subjects. This includes image quality assessment and image averaging to increase SNR. Furthermore, the following six steps have completed for 9 subjects: 1) removal of non-brain signal values from image, 2) magnetic inhomogeneity correction 3) manual labeling of right/left hemispheres, 3) registration to template, 4) manual sulcal delineation, 5) tissue segmentation and 6) thickness measurement. Functional MRI data have been acquired for the same subjects described above. Results from preprocessed data in 4 subjects are displayed in Figure 1.

SDSU: The San Diego site is also involved in the brain imaging project. We have IRB approval for this component, have piloted the procedures and are currently screening subjects for eligibility. Of the 13 children tested using the neurobehavioral test battery, 1 has been scheduled for the mock scanning session and will be scheduled for scanning upon successful completion of that session. Of the remaining children, 6 are ineligible, 6 have not yet been screened. One additional child who is scheduled for neurohavioral testing has been screened and scheduled for the mock session also. Mock scanning sessions are scheduled for 12/8/2008 and 12/9/2008. We also collected MRI data from 43 subjects at the San Diego site during the CIFASD Phase I funding period.

South Africa: Imaging protocols were tested and refined on the Siemens 3T Allegra system housed at the Tygerberg Academic Hospital, Stellenbosch University, Cape Town, South Africa in coordination with Dr. Colleen Adnams (Subcontract PI) and colleagues and Dr. Katherine Narr (co-investigator, Brain Imaging) in February, 2008. The translation of functional imaging stimuli from English to Afrikaans and of supporting experimental materials were subsequently completed during the current budget period. However, due to administrative delays concerning fund transfer for the Neurobehavioral Core of the project, for which subject recruitment and data collection occur in parallel with the Imaging Core, the collection of subject brain imaging (and neurobehavioral) data was postponed during FY1 at the Cape Town site. Project funds for both the Imaging and Neurobehavioral Cores have recently become available and staffing for the relevant project components are now in progress.

To facilitate the collection of test subject data for the Brain Imaging project in FY2, Dr. Narr will visit the Cape Town site in February 2009. Goals for this visit will include (1) the review of imaging protocols with Dr. Adnams and Dr. Meintjies (imaging project collaborator), technical staff and post-doctoral students, (2) testing of the translated functional imaging stimuli in the scanner environment, (3) the collection of human phantom data that is planned to occur at all three CIFASD imaging sites within each budget period, (3) the collection of pilot data from 2-3 healthy control children demographically similar to the FASD group, and (5) the resolution of any other remaining issues relating to the CIFASD imaging experiments. Institution Review Board (IRB) approval for the imaging protocols at the Cape Town site are current and funds are now available for immediate use. Dr. Adnams projects that image data acquisition for FASD study participants will commence in March, 2009 in tandem with data collection for the Neurobehavioral Core. Since the cohort of potential participants will be identified from an existing subject pool, data collection may be accelerated during FY2 to allay initial delays of data collection in FY1.

Neurobehavioral, Facial Imaging and Genetic (saliva) Data Collection:

UCLA staff trained in administration of neuropsychological and parent measures at SDSU in December 2007 and January 2008, and the primary research assistant met reliability standards established by SDSU in May 2008. Subjects began participating in May 2008, and since then seven exposed children (mean age=13.1, 2 females) and two unexposed children (mean age=12.6, 1 female) have participated in the study, including completion of the neuropsychological battery and parent interviews/behavioral measures. In addition, five exposed children and six controls are currently scheduled to participate through February 2009. While no children diagnosed with ADHD have participated yet, active recruitment continues for participants in the ADHD contrast group, and Dr. James McCracken, Director of Child Psychiatry at UCLA, will be assisting with ongoing recruitment efforts. The subject total goal through December 2008 was 12-20 subjects, but delays in subcontract funding impeded progress. With subjects scheduled through February 2009 and continued recruitment efforts in

place, it is expected that UCLA will be at or close to its February 1, 2009 goal of 19-24 subjects. Dr. Ken Jones is scheduled to conduct dysmorphology exams on alcohol-exposed and control subjects on January 15 and 16, 2009, and it is expected that 3-D photographs will also be taken at that time. Given the expected increase in subjects, a new research assistant was hired in July 2008. Dr. Christopher Nuñez, post-doctoral fellow and clinical neuropsychologist, has dedicated significant effort to training the new research assistant on neuropsychological test administration and ensuring that she meets reliability standards. It is also expected that the addition of this staff member will be instrumental in increasing subject totals. Finally, IRB approval has been obtained for collection of saliva samples, and we will begin collecting genetic data for our next subject scheduled for December 4, 2008.

Continuation from Initial CIFASD U24 Imaging Core:

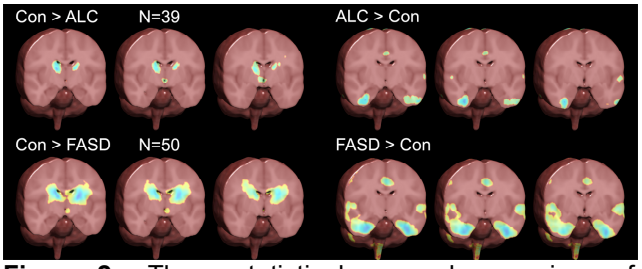


Figure 2: These statistical maps show regions of decreased brain volume in individuals with FASDs relative to controls (on the left), and increased volume in individuals with FASDs relative to controls (on right). In the top row, subjects with facial dysmorphology are excluded, and in the bottom row, all FASD subjects including those with facial dysmorphology are represented).

Since our last progress report presented in June 2008, we have continued to analyze data collected by the brain imaging core from the initial funding period. A new manuscript on the impact of prenatal alcohol exposure on brain activation during verbal working memory has is under review with it's second round of revisions at *Human Brain Mapping* (O'Hare et al., Submitted), and several other papers are in preparation. We have applied new structural image analyses techniques**,** Tensor-based morphometry (TBM), which allows investigations of regional differences in the volumes of brain substructures throughout the entire volume of the brain (including cortical and subcortical gray and white matter) by globally aligning all brain images into a common brain template before applying localized deformations to adjust each subject's anatomy to match the global group-average template. Using these methods, we have observed brain volume decreases in basal ganglia, and brain volume increases in temporal lobes and cingulated cortices bilaterally (see Figure 2).

VI. Discussion:

The studies and results described above illustrate our continued dedication to apply the most advanced brain image analysis tools to the brain imaging data collected by the CIFASD. We have collected and analyzed brain imaging data for 12 subjects at UCLA, and the first subjects are scheduled at SDSU. We will put forth efforts at combining data from the multiple sites to increase sample sizes overall.

VII. Integration 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 scheduled for January 2009 when Ken Jones and Elizabeth Moore are scheduled to evaluate 15 participants from UCLA.

VIII. Plans for the Next Year:

We will continue or begin data collection. Preliminary data analysis should commence.

IX. Publications:

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., (2008) Mapping White Matter Integrity and Neurobehavioral Correlates in Children with Fetal Alcohol Spectrum Disorders. *Journal of Neuroscience*, 28(6):1313-9.

Submitted Manuscripts:

O'Hare E.D., Lu L.H., Houston S.M, Bookheimer S.Y., Mattson S.N., O'Connor M.J., and Sowell E.R. Altered frontal-parietal functioning during verbal working memory in children and adolescents with heavy prenatal alcohol exposure.

X. Posters and Presentations:

O'Hare, ED, Lu, LH, Bookheimer, SY, McCourt, ST, Houston, SM, Mattson, SN, O'Connor, MJ, and Sowell, ER. (2007, November). Increased dorsal frontal and inferior parietal activation during verbal working memory among children and adolescents with prenatal alcohol exposure. Poster presented at the 37th Annual Meeting of the Society for Neuroscience, San Diego, California.

Nuñez, C., Dapretto, M., Lu, L.H., Bookheimer, S.Y., O'Connor, M., Sowell, E.R. (2008). Increased frontal activation during language processing in children with Fetal Alcohol Spectrum Disorders. Poster session submitted to the 38th Annual Meeting of the Society for Neuroscience, Washington, D.C.