Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD)

Progress Report
April 2014

CIFASD Spring 2014 Meeting
Rockville, MD
April 7-8, 2014

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APPENDIX MATERIALS

- Google Data Tables and Graphs (data collected through March 2014)
- CIFASD 2013-2014 Publications

PROGRESS REPORT SUMMARY	GRANT NUMBER 5U24AA014811-11	
	PERIOD COVERED BY THIS REPORT	
PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR Edward P. Riley, Ph.D.	FROM 03/01/2013	THROUGH 03/20/2014

APPLICANT ORGANIZATION
San Diego State University Research Foundation

TITLE OF PROJECT (Repeat title shown in Item 1 on first page)
Administrative Core of the CIFASD

A. Human Subjects (Complete Item 6 on the Face Page)		
Involvement of Human Subjects	☒ No Change Since Previous Submission	☐ Change
B. Vertebrate Animals (Complete Item 7 on the Face Page)		
Use of Vertebrate Animals	☒ No Change Since Previous Submission	☐ Change
C. Select Agent Research	☒ No Change Since Previous Submission	☐ Change
D. Multiple PD/PI Leadership Plan	☒ No Change Since Previous Submission	☐ Change
E. Human Embryonic Stem Cell Line(s) Used	☒ No Change Since Previous Submission	☐ Change

SEE PHS 2590 INSTRUCTIONS.
WOMEN AND MINORITY INCLUSION: See PHS 398 Instructions. Use Inclusion Enrollment Report Format Page and, if necessary, Targeted/Planned Enrollment Format Page.

A. Specific Aims. No modifications have been made since the renewal application (December 2011).

B. Studies and Results. Progress has been made for all CIFASD components and projects as the second year of this renewal segment, Phase III, comes to a close. The Administrative Core has continued to fulfill its role by fostering collaborations, centralizing shared resources, facilitating communication among investigators, ensuring projects are on track to be successful, and educating the public, policy makers, and scientists about FASD and recent CIFASD research findings.

The CIFASD Phase III conference calls continue to be held on the first Wednesday of each month. Voice portions of the calls are conducted through AccuConference. During this reporting period, WebEx has been used to enhance the calls with the sharing of visual information via the internet. For example, during each call, scheduled PowerPoint (PPT) presentations are available for all to view. These PPT presentations allow projects to provide visual updates on their research and receive feedback and guidance from the group. When possible, topic driven themes across projects are scheduled, time permitting, to assist in the facilitation of collaborations between the projects. The conference calls provide all CIFASD investigators with opportunities to discuss both their individual project's goals and those of the CIFASD as a whole. Calls are moderated by Dr. Riley, or in his absence, Drs. Charness or Thomas. The Administrative Core assists the group with the coordination and scheduling of any smaller working group conference calls, as needed. For example, conference calls among select investigators were held to discuss genetic data within CIFASD and possible directions these investigators can explore in the future.

The Informatics Core supplies monthly reports to Dr. Riley charting the data that has been entered into the Central Repository. Spreadsheets and graphs housed within Google Drive illustrate data collection progress for CIFASD clinical projects and are maintained by the Administrative Core. Projects update their completed recruitment and testing numbers on a monthly basis. During this reporting period, the data tables and graphs were modified to show the full 5-year trajectory of each project's aims, as recommended by NIAAA staff. Graphs now illustrate the project's monthly progress in comparison to goals across the project's full timeline to better track data collection success rates.

The Administrative Core coordinated the CIFASD meeting at RSA in Orlando, FL in June 2013 and is currently finalizing the details for the April 2014 face-to-face meeting to be held in Rockville, MD on April 7-8. Arrangements for the meeting include polling for date selection, contracting the sleeping room rates, reserving meeting space, completing travel arrangements for the PI, Scientific Director, Science Advisory

Board and invited guests, and collating and distributing meeting materials. In collaboration with consortium members, the Consortium Coordinator, Dr. Riley invites outside experts as guests to the meeting. Invited guests provide interesting new perspectives to the consortium including new techniques and applications of data that could help CIFASD achieve its goals and move the FASD research field forward. These invited speakers help CIFASD identify new, productive collaborations and/or research directions.

Mid-year progress reports were collected in December 2013 and reviewed by the Science Advisory Board (SAB) composed of the Administrative Core PI, Scientific Director, SAB members and NIAAA staff. These reports along with the NIH progress reports (collected April 1 for each project), data collection charts and graphs (Central Repository and Google Drive), and PPT presentations at the upcoming meeting will all be used to complete the formal evaluations of each project by the SAB. A consensus review will be emailed to each PI.

The Administrative Core continues to update and maintain the CIFASD.org website. During the last reporting period, the Administrative Core facilitated extensive changes to the website to better communicate CIFASD findings to the general public. The home page was improved to be more user friendly and to highlight findings not only by the CIFASD, but the larger fetal alcohol research community. The site now includes more lay descriptions of each CIFASD research project so that our mission and methods are understandable to a broader audience. An Education section of the website was added which includes videos of researchers discussing issues and findings within the field as well as the CIFASD slide set. The slide set was a worthwhile accomplishment of the group in 2013 and is proving to be an excellent resource for the Educational Component. Overall, these modifications of the website allow for the public to be kept abreast of the current state of FASD knowledge. The World Health Organization continues to utilize a secured section of the CIFASD.org website to access CIFASD protocols for their training and data collection. The revamped website also contains a streamlined contact feature that allows researchers to reach out to CIFASD for advice on neuropsychological screening tools and to request for opportunities to be involved in CIFASD projects. The Core's Administrative Specialist, Dr. Thomas, continues to collect news and event items to add to the website and is the liaison between CIFASD and NOFAS.

With the redesign of the website, the Administrative Core modified the Publication section of the website to a PubMed generated listing. This gives the public one-click access to the abstracts of our publications and the free PubMed Central full article versions, as available.

The PI, Dr. Riley, and Scientific Director, Dr. Charness, gave several presentations promoting CIFASD in this reporting period. These national and international presentations provide the consortium with exposure and potential new interactions. Dr. Riley was invited to speak and provide CIFASD updates at several international engagements. In Japan he spoke before the Japanese National Diet, the equivalent of the US Congress, as they considered new laws regulating alcohol policies (these policies have recently been implemented). He also spoke in Windhoek, Namibia at a meeting co-sponsored by the World Health Organization and NIAAA focusing on assessment of FASD. The audience included leading health administrators and physicians from seven African countries. He also presented on CIFASD at the ESBRA meeting in Warsaw, Poland. Stateside, he lectured to pediatric fellows at Sanford Medical Center in Sioux Falls, SD and was able to introduce CIFASD to a broader audience. Dr. Riley continues to serve the community by presenting at many mixed audience conferences. Of particular note was his participation at a camp for adults with FASD and their families just outside of Toledo, OH. Dr. Riley is also the current President of ISBRA and this role allows him to promote CIFASD to the international research community. As Scientific Director, Dr. Charness presented a summary of CIFASD accomplishments to a meeting of the Prenatal Alcohol SIDS and Stillbirth (PASS) Network steering committee at NIAAA and successfully engaged PASS to collaborate with CIFASD on its 3D facial imaging project. He has also spurred studies on the identification of candidate genes nominated from experiments on cellular and animal models of FASD. Dr. Charness also organized a symposium featuring CIFASD studies on genetic susceptibility to FASD, which was accepted as part of the program for the upcoming joint RSA/ISBRA meeting in Bellevue, WA.

The Administrative Core administers the CIFASD developmental projects and oversees the Educational Component. Progress reports for the Educational Component and the developmental projects follow this report. A major accomplishment of the Educational Component has been the roll out of its monthly webinars.

C. Significance. The Administrative Core has helped to facilitate the success of the consortium and its individual projects. It has assisted in the expansion of the consortium by funding developmental projects and providing administrative support to meet the goals of CIFASD. The PI and Scientific Director have increased the exposure of the findings of the CIFASD through their many presentations and the partnership with NOFAS has expanded outreach efforts. The SAB continues to provide oversight and direction for CIFASD.

D. Plans. The Administrative Core will continue its charge to keep all CIFASD projects on target for their data collection goals and encourage the timely publication of their findings by monitoring progress and addressing issues during the monthly conference calls. The next face-to-face meeting will convene in April 2014 and formal project evaluations will be completed by the SAB and sent to each PI. At the start of the new budget period in June 2014, a third developmental project will be added (Dr. Foroud, PI). Solicitation of developmental projects, scheduled to start in June 2015, will begin with announcements at the annual meeting of the Research Society on Alcoholism (RSA) in Bellevue, WA.

E. Publications. A list of publications generated through NCBI can be found on page 32.

F. Project-Generated Resources. N/A

Subcontract Project Title: Educational Component of the Administrative Core of CIFASD
PIs: Tom Donaldson and Kathy Mitchell, National Organization on Fetal Alcohol Syndrome
Reporting Period: April 2013 - March 2014

A. Specific Aims. The primary goal of the CIFASD Educational Component is for the PIs, NOFAS executives, to present both published and unpublished information and data from CIFASD research projects to diverse audiences both nationally and internationally through in-person presentations and multi-media channels. Audiences reached include medical and allied health professionals and students, educators and school administrators, scientists and researchers, and lay audiences including the public at-large. NOFAS also communicates CIFASD published findings to the media and, upon request, to national and state policymakers and public officials.

B. Studies and Results. During this reporting period, NOFAS presented CIFASD information and published findings at numerous events, conferences and meetings. Importantly, CIFASD created a slide set used by the Educational Component PIs to distribute information to numerous audiences. CIFASD project PIs prepared slides and the Administrative Core and Educational Component personnel combined all slides into a fluid presentation complete with notes to be used by NOFAS in their outreach. This slide set is utilized by the Educational Component PIs as they give talks to groups ranging from policy makers to parents to media outlets, and as a result, the work of the CIFASD consortium is shared with new audiences. The slide set was finalized at the June 2013 meeting and posted in the Education section of the revamped CIFASD.org website. NOFAS also kicked off its webinar series in November 2013. This monthly hour-long webinar series is free for anyone to attend and features current FASD research, intervention and treatment strategies, and other topics, presented by leading authorities from the FASD field. The webinar series has already featured three CIFASD investigators and six others are on the upcoming 2014 schedule.

Selections of the Educational Component's outreach during this reporting period include the following:

April 2013
- Presented a workshop for the Missouri Drug Court Annual Conference.
- Presented a plenary for the Indiana Perinatal Task Force conference.
- Facilitated a two-day FASD knowledge and skills training for healthcare professionals from the White Earth Reservation, MN.

May 2013
- Presented a lunchtime keynote for the Alaska RTC at their FASD Conference in Anchorage, AK.
- Provided a three-hour workshop for healthcare providers in Richmond, VA for the Virginia Department of Health and Human Services.

June 2013
- Provided a Grand Rounds for the behavioral psychologists and residents of the Johns Hopkins Kennedy Krieger Institute in Baltimore, MD.
- Presented to 25 NOFAS affiliates at the 2013 NOFAS Affiliate Network Summit.
- Upon request, presented published findings to the staff of Senators Tim Johnson, Lisa Murkowski and Lamar Alexander.

July 2013
- Provided a keynote on FASD and two workshops on FASD and Empowering Women for the Minnesota American Indian Substance Abuse Conference held at the Fond du Lac reservation.
- Upon request, presented published findings to the staff of Senators Mark Begich, Patty Murray, Barbara Boxer, Mary Landrieu, Ron Johnson, Tom Coburn, Sherrod Brown and Tom Harkin.

August 2013
- Provided a workshop for the staff from Crossroads Treatment Center, a large addiction center in Fairfax County, Virginia.
- Presented to the Caron Treatment Centers clinical leadership team to discuss addressing FASD assessment and diagnosis and modification of care within their national network.
- Upon request, presented published findings to the staff of the House of Representatives Energy and Commerce Committee, Health Subcommittee, and Representative Eric Cantor.

September 2013

- Presented *The NOFAS Experience: 25 Years of Policy, Media and Prevention* at the 40th Anniversary of FAS conference in Atlantic City, New Jersey.
- Presented two oral presentations and one panel at the First International Prevention of FASD Conference in Edmonton, Alberta, Canada.
- Upon request, presented published findings to the staff of Representatives Don Young, Frank Pallone and Jim Moran, Senator Dick Durbin, Ohio Governor John Kasich, and state lawmakers in Texas, Virginia, Maryland and Illinois.

October 2013

- Presented to a delegation of visiting government leaders, public health officials, and physicians visiting Washington, D.C. from Guangzhou Municipality in China.
- Presented two Grand Rounds to the medical staff at the Dayton Children's Hospital in Dayton, Ohio.

November 2013

- Presented a plenary for the Virginia Juvenile Justice Conference, Charlotte, VA.
- Hosted a webinar featuring Kathy Mitchell, *Introduction to the Webinar Series and to NOFAS; Developing Resiliency in Family Systems.*

December 2013

- Hosted a webinar featuring Ed Riley, PhD, *The CIFASD: From the Lab to Changing Lives.*

January 2014

- Hosted a webinar featuring Eileen Elias and Doug Waite, MD, *Improving Awareness and Treatment of Children, Adolescents, and Adults with FASD and Co-Occurring Disorders.*

February 2014

- Presented and co-sponsored one-day trainings in Washington, D.C. and Baltimore, MD on FASD and the law with the District of Columbia Public Defender Services, Maryland Disability Law Clinic, Maryland Office of Public Defender, and Morgan State University School of Social Work.
- Hosted a webinar featuring Julie Kable, PhD, *Updates on Neurobehavioral Disorder Associated with Prenatal Alcohol Exposure (ND-PAE).*

March 2014

- Presented a webinar for the Frontier FASD Regional Training Center.
- Hosted a webinar featuring Mary O'Connor, PhD, *New Treatments for Children & Adolescents with FASD.*

C. Significance. NOFAS encourages public health officials and professionals, clinicians, and policymakers to incorporate published findings in health advisories, health promotion initiatives, clinical interventions and policy initiatives. NOFAS assists in bringing the often complex objectives and characteristics of the consortium's basic, biomedical and clinical research to diverse audiences, including individuals living with FAS and their families, in a clear and comprehensible manner helping to promote the rationale for CIFASD's important research.

D. Plans. NOFAS plans to continue presenting and promoting CIFASD information and data to its audiences and enhancing the consortium's outreach by facilitating the monthly one-hour webinars featuring CIFASD researchers; increasing the consortium's presence on NOFAS.org and NOFAS social media, including videotaped interviews with CIFASD scientists; and representing CIFASD through social media outreach, such as Facebook, Twitter and YouTube.

E. Publications. None

F. Project-Generated Resources. None

Development Project Title: Genetic Approaches to Understand Variability in FASD Facial and Neural Phenotypes
PI: Johann K. Eberhart, The University of Texas at Austin
Reporting Period: March 2013 - March 2014

A. Specific Aims. The major goals of this developmental project are to: 1) characterize ethanol-sensitive mutants from a forward genetic screen and 2) screen community resources to identify ethanol-sensitive loci involved in facial or neural development. These goals have not changed.

B. Studies and Results. For Aim 1, we plan to use a whole genome sequencing approach to identify the causative lesions in each forward genetic mutant. To this end, we have generated AB/Tu hybrid mapping crosses for all the forward genetic mutant lines. AB is the genetic background in which the mutagenesis took place and Tu is the reference genome that we will use for mapping. Two mutants are currently in the queue for sequencing. Once we obtain the sequences, they will be concatenated and mapped using Megamapper on the TACC supercomputer. We have hybrid adults for the remaining fish lines.

Our initial screen in Aim 2 has been published. We screened a total of 20 mutants for sensitivity to ethanol and found 5 that interacted with ethanol. Ethanol exacerbated the craniofacial phenotypes of 4 mutants: *hinfp*, *foxi1*, *mars* and *plk1*. Ethanol also induced axon defects in *foxi1* and *plk1* mutants. Ethanol exposed *plk1* mutants had a substantial increase in the levels of apoptosis throughout the embryo. Ethanol interacted synergistically with *vangl2*, a member of the planar cell polarity pathway, to cause synophthalmia, midline craniofacial defects and axon path finding defects.

Based on the strength of the *vangl2*-ethanol interaction, we explored if there was evidence for interactions between ethanol and members of the planar cell polarity pathway in humans. In collaboration with Dr. Foroud, we have found support for interactions between ethanol and several planar cell polarity pathway members, including *VANGL1, CELSR2* and *FAT4*. Of these, the SNP in *FAT4* results in an Alanine to Valine missense mutation. In the absence of alcohol exposure, this minor allele associates with reduced lower facial depth. While alcohol exposed children homozygous for the major allele also have reduced facial depth, alcohol-exposed children with the minor allele have a facial depth similar to that of unexposed children homozygous for the major allele. This intriguing finding may suggest that the minor allele is more resistant to the effects of ethanol. Consistent with the model, this same mutation has been associated with a decreased risk of alcohol-associated cancer.

We are continuing our analyses of gene-ethanol interactions using our previous results to inform our screens. Our results and the human dataset suggest that the planar cell polarity pathway may be broadly sensitive to ethanol teratogenesis. In a previous genetic screen, we recovered two complementing alleles, *b1101* and *b1198*, whose phenotypes suggest that they may play a role in the planar cell polarity pathway. We have screened *b1198* and found that ethanol greatly exacerbates the mutant phenotype.

C. Significance. Our findings provide direct evidence of the genes that regulate sensitivity to ethanol teratogenesis. The zebrafish provides a model system in which to rapidly identify these susceptibility loci. Through continued interactions with other CIFASD members, we will be able to leverage these results to identify those gene-ethanol interactions most likely to mediate sensitivity to FASD. Once identified, the zebrafish model system also provides an excellent system in which to determine the mechanism of gene-ethanol interactions as we have shown in our recent *Development* publication.

D. Plans. For Aim 1, we will sequence the remaining forward genetic mutants. We will validate the genetic lesion using morpholino phenocopy and complementation with existing mutants, where possible. As time and funds allow, we will also characterize the mechanism of the gene-ethanol interaction. These characterizations will focus on the genesis of the craniofacial defects in each mutant. We will analyze neural crest distributions to determine when craniofacial development is first disrupted. We will also characterize the expression of critical signaling molecules in the endoderm and oral ectoderm that guide craniofacial development. Our priorities will be guided by evidence for conservation in human from Dr. Foroud's dataset and by the novelty of the mutation.

In Aim 2, we will pursue follow-up experiments on the interaction between ethanol and the planar cell polarity pathway. We will screen *b1101* for ethanol sensitivity and further characterize the window of ethanol sensitivity

for *b1101*. Those mutants that interact with ethanol will be crossed to Tu to generate a mapping hybrid line for sequencing. Mapping will be performed as for those mutants in Aim 1.

We will also expand our analysis of the planar cell polarity pathway by obtaining curated mutant resources. Many members of the planar cell polarity pathway have been mutated in zebrafish. Because of the human data, we have already obtained *fat4* mutants. We will test if *fat4* mutation sensitizes zebrafish to ethanol-induced craniofacial defects. We will also obtain *vangl1* and *celsr2* mutants to test if they are ethanol-sensitive. Additional planar cell polarity pathway mutants will be selected for analyses based on available and evidence for interaction with ethanol in humans.

The planar cell polarity pathway has known roles in neural development and axon path finding. In our recently published screen, we discovered axon projection defects in ethanol-treated *vangl2* mutants. To further analyze these defects we are generating *vangl2;isl1:EGFP* transgenics to aid in characterizing cranial motor neuron axon guidance in ethanol treated mutants. We will use confocal time-lapsed analyses to trace the trajectory and rate of axon extension in ethanol-treated mutant and control embryos.

E. Publications. Included in the list of publications generated through NCBI on page 32.

F. Project-Generated Resources. None

Development Project Title: Circadian Genes, Stress Axis and Fetal Alcohol Spectrum Disorder
PI: Dipak K. Sarkar, Rutgers
Reporting Period: March 2013 - March 2014

A. Specific Aims. The major goal of this project is to measure Per genes methylation and gene expression as well as plasma cortisol levels using the DNA and plasma samples of FASD and non-FASD patient children from the CIFASD projects using a structured protocol for diagnosis of FAS using the cardinal facial and growth features collected from and/or South African populations. Once available, this project will compare the gene and hormone data of subjects classified as FAS, deferred (some characteristic features of FAS), or no FAS. Individuals with FAS, deferred and no FAS will be longitudinally assessed, with genotypes and phenotypical characterization for a period of 3 years. The questions to be addressed here are: Do methylation and expression levels of *Per* genes correlate with the stress abnormality (high cortisol)? Can these methylation sites and patterns be used as biomarkers that predict future endophenotypes of stress abnormalities (e.g., anxiety, alcohol binging or immune abnormalities)? And furthermore, if *Per* genes hypermethylation is involved in stress abnormalities, is this manifested in patients with FASD? These studies will be crucial in determining the stability of identified epigenetic signature of FASD patients and whether epigenetic signatures identified allow for earlier identification of alcohol-related birth outcomes.

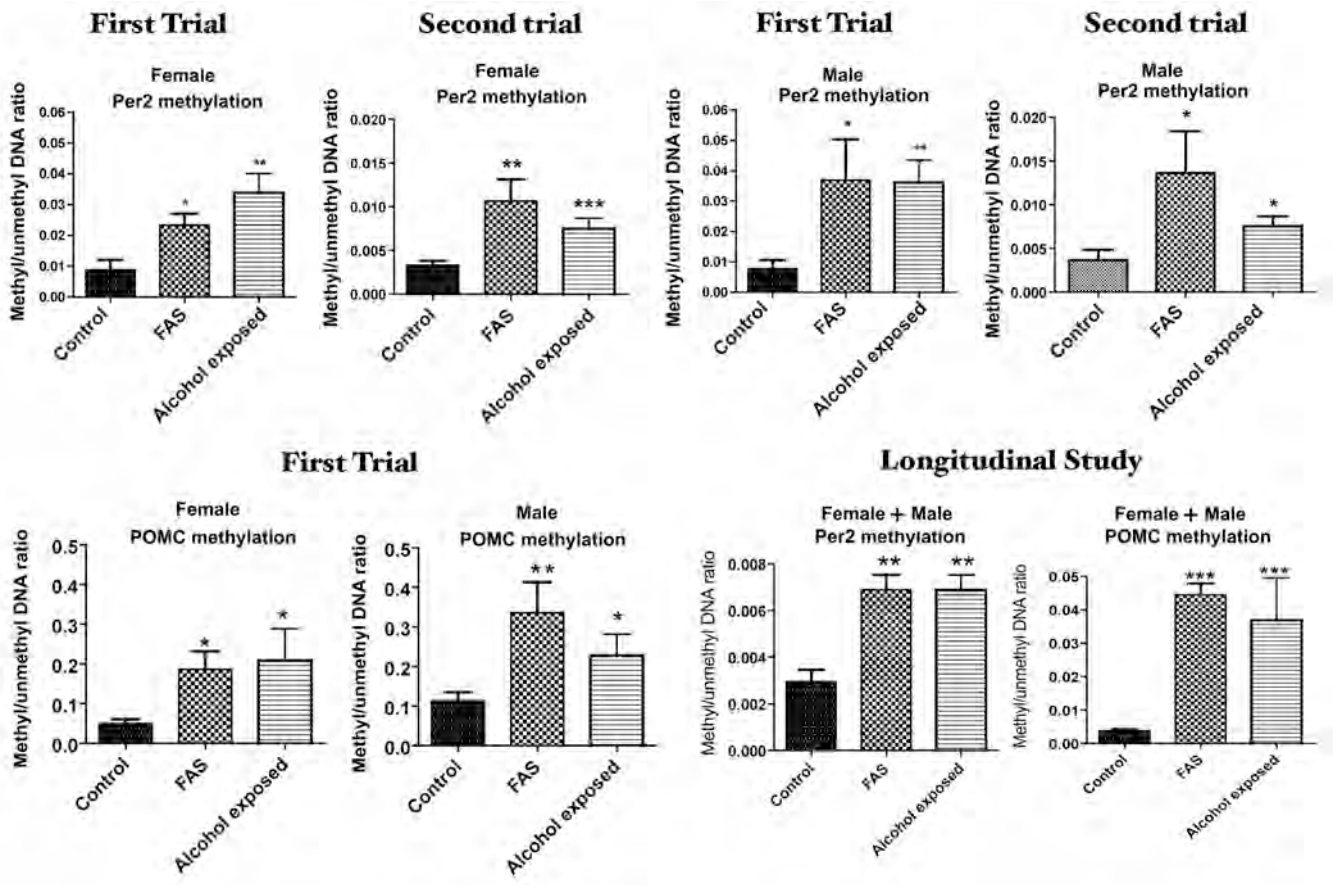
B. Studies and Results. This project works with other Consortium project personnel including Drs. Christina Chambers, Tatiana Foroud, Elizabeth Sowell, Sarah Mattson, Jeff Wozniak, and Claire Coles. We received initially 90 frozen salivary DNA samples (about 4 microgram each) from Dr. Foroud's laboratory at Indiana University. According to information provided by Dr. Foroud, these samples were obtained from Caucasian (either confirmed by GWAS or self-report when GWAS was not available) female and male children of the United States CIFASD cohort. These samples included 18 female and 29 male controls, 7 female and 6 male FAS subjects (as classified by the Dysmorphology Core) and 8 female and 19 male prenatal alcohol exposed subjects. We performed DNA bisulphite modification and measured the CT values of the DNA. After confirming the quality of the DNA, we used them in methylation assay for Per2 gene and POMC gene.

We measured Per gene and POMC gene methylation by SYBR Green Methylation-specific (MSP) Real-Time PCR. DNA samples were undergone through the bisulphate conversion and performed with the help of EZ DNA Methylation-Direct Kit (Zymo Research, Inc.). PCR primers were designed using the MethPrimer program for human (http://www.urogene.org/methprimer/index1.html). Primers were designed to be methylation-specific or unmethylation-specific with respect to the particular cytosine nucleotides in the CpG pair under analysis. The ratios of the methylation-specific to unmethylation-specific responses were quantified by ΔCt method. The Real-Time PCR was run on ABI Prism 7500 Sequence Detection System (Applied Biosystems, Inc.) using PowerGreen PCR Master Mix (Applied Biosystems, Inc.). 2-4 µl of bisulfate-treated DNA was utilized in each run. All runs were performed in duplicate.

In early January 2014, we received a second batch of 268 salivary DNA samples from Dr. Foroud's laboratory at Indiana University. These samples included 61 female and 77 male controls, 8 female and 6 male FAS subjects, and 35 female and 54 male prenatal alcohol exposed subjects. Of these, we received 16 DNA samples from the same patients that were used in the first trial for conducting longitudinal studies. So far we have completed measurements of Per2 and POMC DNA methylation of the samples for longitudinal studies and Per2 DNA methylation of the rest of the samples.

The methylation analysis of Per2 and POMC genes of salivary DNA samples are completed and the data are shown in the following figure. As can be seen, Per2 and POMC gene methylation levels are significantly higher in both male and female FAS and alcohol exposed subjects, as compared to control subjects in the first trial. As in the first trial, Per gene methylation levels showed similar trend of increased methylation in both FAS and alcohol exposed male and female subjects. Determination of a limited number of samples in a longitudinal study has also shown that increased Per2 and POMC methylation persisted in the same patients in a follow-up study.

C. Significance. Because clinical sleep-wake disturbances have been observed in human neonates, children, and adolescents following prenatal exposure to alcohol, we have previously used a rat model to determine whether prenatal ethanol exposure produces long-term alterations in the circadian clock mechanism to effect the circadian regulation of endocrine functions. We found that prenatal ethanol exposure produced long-term changes in the pattern of the circadian rhythms of POMC and the clock-governing rat *Per* genes in the arcuate nucleus and the *Per* genes in the suprachiasmatic nucleus of the hypothalamus.



Prenatal ethanol exposure has been shown to alter core body temperature and phase shifting ability, glucocorticoid rhythms, immune cell rhythms, and rhythmic pituitary-adrenal function. These data provide evidence for the involvement of *Per2* in the mechanism involved in fetal alcohol altered stress control. Epigenetic modification of a gene has been shown to play a role in maintaining a long-lasting change in gene expression. Therefore, we hypothesize that alcohol's modulating effect on DNA methylation makes an epigenetic mark on *Per* genes that serves to activate the stress axis via suppression of the POMC gene leading to maladaptation of the stress system. Hence, we anticipate a significant association between *Per2* gene methylation and stress hormones (cortisol) abnormality in FASD patients. The data shown above provide exciting preliminary evidence that prenatal ethanol makes an epigenetic mark on *Per2* and POMC genes in human patients.

D. Plans. We now need to show that the methylation marks on *Per2* and POMC genes are also detectable in blood DNA samples. Further longitudinal studies need to be conducted for few more samples (since the number in patients we used in the study we already conducted were only between 3 and 10/group). Additionally, we need to finish POMC DNA gene methylation in the rest of the samples we currently have. We also are measuring site specific methylation changes on the gene (e.g., pyrosequencing to better defining which part of the *Per2* and POMC DNAs getting hypermethylated) and control gene studies (e.g., doing other clock gene *Per*1 or CNS specific genes to determine the specificity). Furthermore, we need to test whether *Per2* and POMC gene methylation and expression levels correlate with the stress abnormality (high cortisol and testosterone). These studies will be crucial in determining whether epigenetic signatures identified allow for earlier identification of alcohol-related birth outcomes.

E. Publications. Included in the list of publications generated through NCBI on page 32.

F. Project-Generated Resources. None

Development Project Title: Craniofacial Effects of Prenatal Alcohol Exposure in 3D Fetal Ultrasound Images
PI: Alison Noble, Oxford University
Reporting Period: December 2013 - March 2014

A. Specific Aims. The specific aims for this developmental project are as follows:

1. To determine if the "3D" curve of the face profile is reliably segmentable.
2. To identify variation in the quality of face surfaces segmentable from 3D U/S images.
3. To determine which facial regions produce shape models good enough for face discrimination studies.

B. Studies and Results. This development project involves three collaborating partner institutions: Professor Alison Noble and Tom Rackham at Oxford University; Professor Peter Hammond (CIFASD PI) and Mike Suttie at University College of London; and Professor Hein Odendaal and Rosemary Meyer at University of Stellenbosch in South Africa who are involved in the PASS project.

Due to delays in formalizing the transfer of funds to Oxford University, recruitment of Tom Rackham, a DPhil candidate of Professor Noble's, was not possible until December 2013. Work on the project began in January 2014. As such, this report covers a period of research activity of approximately 10 weeks in which only specific aim 1 has been addressed. This aim is to examine the segmentation of the fetal profile from 3D ultrasound (U/S). Collaborators at Stellenbosch University previously provided 24 3D U/S fetal images taken at 28 weeks gestation. Analysis began with the segmentation of the mid-line facial profile (Figure 1) using the following protocol:

A. Feature Symmetry [1] is used to identify ridges in the ultrasound image.
B. Local Energy is combined with Feature Symmetry to find high intensity ridges (Fig. 1A) and hence segment the skull (Fig. 1B).
C. The orientation of the skull is used to rotate the image such that the face lies on the x-axis (Fig. 1C).
D. Feature Asymmetry [1] is used to identify edges in the rotated image (Fig. 1D). Only the largest connected region where Feature Asymmetry>0 is considered.
E. Local Orientation is used to identify the orientation of edges detected in Fig. 1D. Fig. 1E shows regions where Local Orientation>0 in green and Local Orientation<0 in red.
F. Regions where there is a Feature Asymmetry response (indicating an edge) and the Local Orientation is greater than zero (indicating an upwards facing edge) are preserved (Fig. 1F).

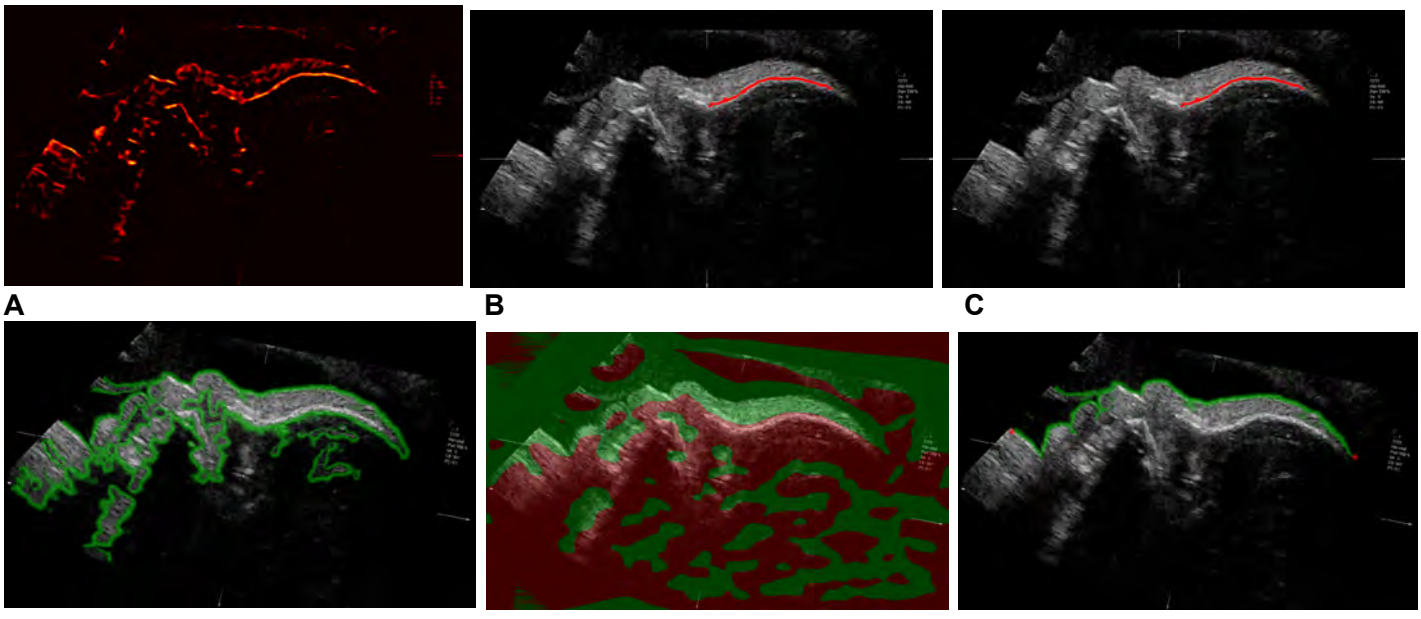


Figure 1 Image Alignment and Fetal Profile Segmentation

Reference [1]: Kovesi, P. Symmetry and asymmetry from local phase. *Tenth Australian Joint Conference on Artificial Intelligence*, 1997: Vol. 190.

The same protocol was applied to a series of 3D U/S images to demonstrate the variation in the fetal facial profile that is segmentable (Figure 2).

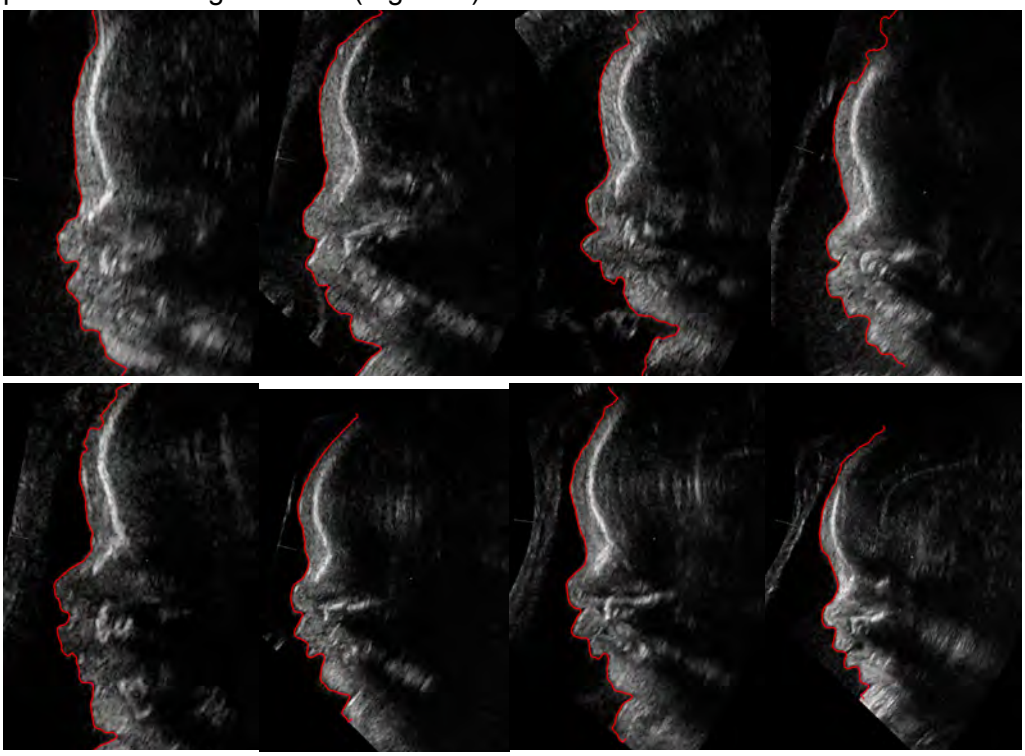


Figure 2 Examples of Segmented Fetal Facial Profiles

C. Significance. In a previous CIFASD study of a Cape-Colored cohort of controls and children exposed prenatally to alcohol (Suttie, et al., 2012), the mid-line facial profile was shown to be almost as effective as the full face in discriminating between controls and children with an FAS diagnosis. Therefore, the mid-line facial profile of a developing fetus has the potential to assist the recognition of the effects of prenatal alcohol exposure.

D. Plans. 3D facial photographs of the PASS cohort are also captured at one month and one year. These are already being analyzed at the UCL Institute of Child Health and have proven effective in identifying facial features associated with prenatal alcohol exposure. In order to analyze surface face features, it will be advantageous if surface points on the fetal face surface can be extracted from the 3D U/S. Therefore, in the future, we will consider the possibility of segmenting the point cloud making up the fetal face surface and mixing face surfaces segmented from 3D U/S and those captured by 3D facial photogrammetry to build a dense surface model of face shape covering the period 28 weeks preterm to one year of age.

E. Publications. None

F. Project-Generated Resources. None

Publications Reported for this Reporting Period

NIH Public Access Compliance	Citation
Complete	Dou X, Wilkemeyer MF, Menkari CE, Parnell SE, Sulik KK, Charness ME. Mitogen-activated protein kinase modulates ethanol inhibition of cell adhesion mediated by the L1 neural cell adhesion molecule. Proc Natl Acad Sci U S A. 2013 Apr 2;110(14):5683-8. PubMed PMID: 23431142; PubMed Central PMCID: PMC3619378.
Complete	Eberhart JK, Harris RA. Understanding variability in ethanol teratogenicity. Proc Natl Acad Sci U S A. 2013 Apr 2;110(14):5285-6. PubMed PMID: 23513226; PubMed Central PMCID: PMC3619315.
Complete	Glass L, Ware AL, Crocker N, Deweese BN, Coles CD, Kable JA, May PA, Kalberg WO, Sowell ER, Jones KL, Riley EP, Mattson SN, Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD). Neuropsychological deficits associated with heavy prenatal alcohol exposure are not exacerbated by ADHD. Neuropsychology. 2013 Nov;27(6):713-24. PubMed PMID: 24040921; PubMed Central PMCID: PMC3898510.
Complete	O'Brien JW, Norman AL, Fryer SL, Tapert SF, Paulus MP, Jones KL, Riley EP, Mattson SN. Effect of predictive cuing on response inhibition in children with heavy prenatal alcohol exposure. Alcohol Clin Exp Res. 2013 Apr;37(4):644-54. PubMed PMID: 23094678; PubMed Central PMCID: PMC3771541.
Complete	Rachdaoui N, Sarkar DK. Effects of alcohol on the endocrine system. Endocrinol Metab Clin North Am. 2013 Sep;42(3):593-615. PubMed PMID: 24011889; PubMed Central PMCID: PMC3767933.
In process at NIHMS	Swartz ME, Wells MB, Griffin M, McCarthy N, Lovely CB, McGurk P, Rozacky J, Eberhart JK. A screen of zebrafish mutants identifies ethanol-sensitive genetic Loci. Alcohol Clin Exp Res. 2014 Mar;38(3):694-703. PubMed PMID: 24164477; NIHMSID: 527375.

A. Specific Aims

The specific aims have not been modified.

B. Studies and Results

The following specific aims were addressed in this period:

Aim 1.1: The Informatics Core will continue to provide data management and integration and will lead the ongoing development of the CIFASD data dictionary, architectures for managing CIFASD data, and tools for data quality improvement and exploratory data analysis.

- The Tallies Completeness Report had the following enhancements and modifications:
    - Addition of Final Tallies, Tallies %, and Historical Tallies reports
    - Inclusion of Demographics Phase III data.
    - Changes in the VASBIIP report.
- The DemGroupClass Report was modified to reflect for Neuro III Time Tested I data.

Aim 1.2: It will continue to provide reporting support to the Administrative Core to track consortium progress.

- The CIFASD monthly data report continues to inform the Administrative Core about the progress that has been made regarding uploading data in the Central Repository.
- The Informatics Core leads the CIFASD Data Access Committee, charged with developing and implementing policies, procedures, and methods to share CIFASD data beyond the consortium.

Aim 2.1: The Informatics Core will create additional software tools to incorporate new datasets for modalities that are already present in the Central Repository.

- Support for Eating Habit Interview Data (data dictionary definition, creation of input tool, upload into the Central Repository, and data retrieval) was added.
- Changes in the Infant Neurobehavior [Bayley, Maternal Questionnaire, and Heart Rate Monitoring] upload and duplicate checking to accommodate multiple AR and NPSY records.
- Creation of new and extended existing Preschool Neurobehavior datasets to accommodate new requirements for age and new fields.
- Finish Data Dictionary and MS Access Input Tool for Follow-up/Outcome (2nd Interview) to accommodate requirements for multiple birth capture.
- Changes in the Data Dictionary, MS Access Input Tool, and upload and duplicate checking for Alcohol & Control (EEAC) to accommodate requirements for Biospecimen data.
- Provided batch import and processing for Biospecimen dataset.
- Modified Neurobehavior Phase III (data dictionary definition, creation of input tool, upload into the Central Repository, and data retrieval) to support several new datasets to accommodate data potentially useful for PreNeuro datasets. Changed import function for CANTAB, CBCL, CVLT, Conners, BRIEF, and NEPSY to provide batch processing. Corrected value ranges and data types for multiple tables based on feedback from consortium members.

Aim 2.2: The Informatics Core will modify existing, or develop new, technology solutions to create additional software tools to incorporate datasets for entirely new modalities of data.

- Implementation of a mechanism to share GWAS data.

C. Significance

There are no significant findings to date.

D. Plans

Based on guidance from the project PIs and contingent on their changing research needs, the following are planned for year three:

• Continue providing maintenance, changes, and enhancements to various software tools, particularly Phase III data.
• Provide custom datasets, as requested.
• Provide DGC, Dysmo, Brain and 3D Face Imaging tables in relation to Neuro Phase data by implementing multiple-datasets query.
• Provide a way to record that a 3d facial image has been collected, regardless of whether or not it was measured.
• Lead the development of a data sharing process to allow researchers outside of CIFASD a mechanism to access CIFASD data.

E. Publications

n/a

F. Project-Generated Resources

In accordance with the CIFASD Data Sharing plan, the Central Repository is being maintained and extended to store the data that will be provided under future data-sharing agreements.

Data dictionaries and Input Tools are available to the public from the Central Repository downloads page: https://cifasd.uits.iu.edu/downloads/downloads.html

PROGRESS REPORT SUMMARY	GRANT NUMBER 5 U24 AA014815-11	
	PERIOD COVERED BY THIS REPORT	
PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR Kenneth Lyons Jones	FROM 06/01/2013	THROUGH 05/31/2014

APPLICANT ORGANIZATION
The Regents of the Univ. of Calif., U.C. San Diego

TITLE OF PROJECT (Repeat title shown in Item 1 on first page)
Dysmorphology Core

A. Human Subjects (Complete Item 6 on the Face Page)				
Involvement of Human Subjects	☐	No Change Since Previous Submission	☐	Change
B. Vertebrate Animals (Complete Item 7 on the Face Page)				
Use of Vertebrate Animals	☐	No Change Since Previous Submission	☐	Change
C. Select Agent Research	☒	No Change Since Previous Submission	☐	Change
D. Multiple PD/PI Leadership Plan	☒	No Change Since Previous Submission	☐	Change
E. Human Embryonic Stem Cell Line(s) Used	☒	No Change Since Previous Submission	☐	Change

SEE PHS 2590 INSTRUCTIONS.

WOMEN AND MINORITY INCLUSION: See PHS 398 Instructions. Use Inclusion Enrollment Report Format Page and, if necessary, Targeted/Planned Enrollment Format Page.

Progress Report for Dysmorphology Core
(April 1, 2014)

A.Specific Aims

Aim #1: To assure consistency as well as accuracy in recognition of of Fetal Alcohol Spectrum Disorders (FASD) at all CIFASD project sites throughout the world.

Aim #2: To develop a training DVD that could be used to teach physicians and other health care professionals with little or no experience in diagnosis of FASD to correctly identify the characteristic structural features of FAS through a physical examination and to successfully diagnose or rule out this disorder on that basis.

Aim #3: To develop a methodology whereby long-distance consultation can be provided to physicians and other health care providers in outlying areas throughout the world.

Aim #4: To document the prevalence of major malformations in children prenatally exposed to alcohol, and in so doing, delineate the extent of Alcohol Related Birth Defects (ARBD).

B. Studies and Results

Re specific Aim#1, overall the Dysmorphology Core has performed 3273 standardized physical examinations on 2264 unique subjects at consortium sites throughout the world. 285 of those children have been examined since April 2013. That has included 55 children in Atlanta; 15 children in Los Angeles; 144 children in Minneapolis; 56 children in San Diwgo;14 children in Khmelnytsky and one child in Rivne in Ukraine.

Re Specific Aim #2, we have developed a 20 minute DVD of a normal neonate and a 20 minute DVD of a 6 year old normal child that demonstrates a careful Dysmorphology examination that focuses on minor malformations in structural development. Having shown that this can be developed and that it is effective in training Pediatric residents, we have developed a DVD of a 2 year old child with FAS and a 4 year old with FAS which focuses entirely on minor malformations that are diagnostic of FAS.

Re specific Aim #3, we have had extensive discussions with individuals at the Telecommunications Center at UCSD as well as those in Ukraine. In addition, we were able to set up the basic network and system connectivity at one site in Ukraine to test components of the Phase I Pilot Consultations. (see D. Plans).Unfortunately, following discussions about various alternative strategies necessitated by the need to adapt to various geographic locals, government agencies, individuals and population densities in Ukraine, as well as serious problems with internet and network capabilities and costs associated with importing telemedicine equipment into Ukraine, we have concluded that collaboration on this specific aim with one of the Ukrainian sites will not be possible and that collaboration with the other site will be extremely difficult, We have therefore established a collaboration with Dr Jeffrey Wozniak at the University of Minnesota to carry out this specific aim.

C. Significance

All projects within all sites of CIFASD are completely dependent on Specific Aim #1 of the Dysmorphology Core. Without a firm diagnosis of FAS or Alcohol Related Neurodevelopment Disorder (ARND) , it is not possible to carry out any of the clinical projects in CIFASD.

The majority of physicians throughout the world do not know how to correctly diagnosis FAS. Clearly there are not sufficient dysmorphologists with expertise in diagnoses of FAS to train sufficient numbers of physicians without such expertise. The development of training DVDs that can be viewed by physicians without expertise will help to alleviate this situation throughout the world.

The deficiency of physicians lacking in expertise to diagnose FAS leads to a situation in which children in remote areas throughout the world are unable to get an accurate diagnoses of FAS. By establishing a telemedicine communication system, it will be possible for a physician located in a central area with expertise in diagnosing FAS to provide accurate diagnoses to physicians caring for children in remote areas.

D. Plans

Over the next year, we will continue to see children at all CIFASD sites

We will develop DVDs of eight additional children and adolescents affected with FAS.

In May, IT people from our division will meet with IT people from the University of Minnesota in Minneapolis to make plans about establishing our telecommunication system between the University of Minnesota and UCSD. This change in plans was necessitated because of a number of problems establishing a telecommunication infrastructure in Ukraine. (see B. Studies and Results) We think that the University of Minnesota will be a far better site to develop this system for a number of reasons.

Consultations at University of Minnesota will be performed in collaboration with Jeffrey Wozniak, PhD, LP in Child & Adolescent Psychology who I have been working with over the last two years in that he has a CIFASD site. I have already trained a pediatrician at his site who I have worked with extensively as it relates to children with FAS. We believe we can do this with existing internet and network services on the University of Minnesota campus. In addition, Finally, there is a 2 hour time difference between San Diego and Minneapolis opposed to a 10 hour time difference between San Diego and Ukraine

We will develop and implement the tele-communication system at the University of Minnesota in the following two consultation functional phases.

Phase I: Pilot Consultations: The remote physicians will have the capability to film the physical exam and securely transmit them via the internet to Dr. Jones for review and schedule a consultation. For this consultation, the remote clinicians will be looking at the participant documents and video at their site and Dr. Jones will be looking at the same information on the secure repository at his site.
Phase II: Real-Time Consultations: This phase will give physicians and healthcare providers the capability to perform the clinical examination and consultation with Dr. Jones during a live session at the remote site. Dr. jones will be viewing the participant and consulting with the clinicians to make the diagnosis of FAS or successfully rule out this disorder. Real-time consultations will give clinicians in the remote sites the benefit of interactive consultation with the participant present. The hope is that this will be an effective clinician training tool and a more effective way to determine the next steps for the participants and their families.

E. Publications

NIH Public Access Compliance	Citation
In process at NIHMS	Glass L, Graham DM, Deweese BN, Jones KL, Riley EP, Mattson SN. Correspondence of parent report and laboratory measures of inattention and hyperactivity in children with heavy prenatal alcohol exposure. Neurotoxicol Teratol. 2014 Mar-Apr;42:43-50. PubMed PMID: 24512965; NIHMSID: 570774.
Complete	Molteno CD, Jacobson JL, Carter RC, Dodge NC, Jacobson SW. Infant emotional withdrawal: a precursor of affective and cognitive disturbance in fetal alcohol spectrum disorders. Alcohol Clin Exp Res. 2014 Feb;38(2):479-88. PubMed PMID: 24033350; PubMed Central PMCID: PMC3872495.
Complete	Glass L, Ware AL, Crocker N, Deweese BN, Coles CD, Kable JA, May PA, Kalberg WO, Sowell ER, Jones KL, Riley EP, Mattson SN, Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD). Neuropsychological deficits associated with heavy prenatal alcohol exposure are not exacerbated by ADHD. Neuropsychology. 2013 Nov;27(6):713-24. PubMed PMID: 24040921; PubMed Central PMCID: PMC3898510.
Complete	O'Brien JW, Norman AL, Fryer SL, Tapert SF, Paulus MP, Jones KL, Riley EP, Mattson SN. Effect of predictive cuing on response inhibition in children with heavy prenatal alcohol exposure. Alcohol Clin Exp Res. 2013 Apr;37(4):644-54. PubMed PMID: 23094678; PubMed Central PMCID: PMC3771541.

Program Director/Principal Investigator (Last, First, Middle): Jones, Kenneth Lyons

Inclusion Enrollment Report

This report format should NOT be used for data collection from study participants.

Study Title: Dysmorphology Core

Total Enrollment: 2,264 **Protocol Number:** 071586

Grant Number: 5 U24 AA014815-11

PART A. TOTAL ENROLLMENT REPORT: Number of Subjects Enrolled to Date (Cumulative) by Ethnicity and Race				
Ethnic Category	**Females**	**Males**	**Sex/Gender Unknown or Not Reported**	**Total**
Hispanic or Latino	54	88		142 **
Not Hispanic or Latino	875	930		1,805
Unknown (individuals not reporting ethnicity)	153	164		317
Ethnic Category: Total of All Subjects*	1,082	1,182		2,264 *
Racial Categories				
American Indian/Alaska Native	35	44		79
Asian	10	7		17
Native Hawaiian or Other Pacific Islander	1	0		1
Black or African American	314	324		638
White	572	624		1,196
More Than One Race	39	55		94
Unknown or Not Reported	111	128		239
Racial Categories: Total of All Subjects*	1,082	1,182		2,264 *

PART B. HISPANIC ENROLLMENT REPORT: Number of Hispanics or Latinos Enrolled to Date (Cumulative)				
Racial Categories	**Females**	**Males**	**Sex/Gender Unknown or Not Reported**	**Total**
American Indian or Alaska Native	0	4		4
Asian	1	0		1
Native Hawaiian or Other Pacific Islander				
Black or African American	6	8		14
White	37	59		96
More Than One Race	9	14		23
Unknown or Not Reported	1	3		4
Racial Categories: Total of Hispanics or Latinos**	54	88		142 **

* These totals must agree.
** These totals must agree.

A. Specific Aims

Aim 1 Determine if beneficial effects of a prenatal MVM supplementation intervention in alcohol-exposed children persist at preschool age.
Aim 2 Determine if prenatal alcohol exposure in the context of the prenatal nutritional environment affects child nutritional status and determine the extent to which nutritional status in children prenatally exposed to alcohol affects growth and performance.
Aim 3 Determine if a miRNA biomarker for alcohol exposure previously identified in a shepp model translates to the human as a marker of recent or distant exposure to various levels of alcohol
Aim 4 In collaboration with CIFASD investigators, explore selected genetic and epigenetic risk factors for FASD, the effect of nutrition on 3D facial images, and telemedicine approaches to early diagnosis.
The major goals have not changed since the initial competing award or previous report.

B. Studies and Results

Aims 1 and 2: Recall children from Phase II for 2 and 3.5-5 year old visits; enroll new pregnant women.

Since the project renewal, we have completed the following number of study visits (Table 1).

Table 1. Study visits completed by type: number of visits and number of unique subjects

Visit Under Phase III Protocol	Number of Visits	Number of Unique Subjects
New pregnant women enrolled Phase III	137	137
Mothers re-consented in Phase III for new data collection	42	42
Dysmorphology exams completed	325	223
Prenatal ultrasounds completed	240	139
Cardiac orienting paradigm completed	205	113
3D images captured	72	72
2 year old visits completed	17	17
3.5-5 year old visits completed	25	25

We have enrolled > the target sample of 120 new mothers in the study. We have evaluated 42 children at the 2-year old or 3.5-5-year old visits. The competency and confidence of the testers on the preschool testing battery has been a challenge, and this has been addressed during this project year by in-person training, and weekly/monthly training and review sessions via skype. Maternal interviews, 24-hour dietary recall forms, growth measurements and eating and sleep habit questionnaires have been collected. Biological samples have been collected from mothers and children. The cardiac orienting response protocol has been expanded to both performance sites and now includes auditory/visual protocols for preschool children. The CIFASD Repository database has been finalized to accept these new measures. During the period under review, Dr. Keen's lab has finished analysis of all 1,077 samples from the 10 shipments for Zn, Cu, Fe, Mg, Ca, transferrin receptor and ceruloplasmin activity. Samples through shipment 9 have been analyzed for homocysteine, C-reactive protein, ferritin and vitamin D. We are in the process of analyzing the remaining samples for choline/choline metabolites, folate and vitamin B12. However, the choline and metabolites analyses have been completed for 802/1077 samples (74.5%).

Aim 3: miRNA

A total of 142 maternal plasma samples (71 collected at enrollment, and 71 collected in the 3rd trimester from the same mothers) for the miRNA study by Miranda have been selected from the

pool of archived samples, and these have been sent to Dr. Miranda's lab at Texas A&M.
Initial quality control assessment of samples has been performed for 60 plasma samples and deemed adequate. Dr. Miranda's lab has isolated RNA from a total of 24 samples and has been able on average to recover ~1000 ng RNA per sample. There are no statistically significant differences in RNA recovery due to recruitment site, stage of pregnancy or treatment group.

Aim 4: Sequencing and Methylation Status/3D Images/Telemedicine

500 archived samples of maternal white cells have been received at UCSD and are stored in Dr. Kelly Frazer's lab at the UCSD Institute for Genomics. As there is concern about batch effects, the processing of these samples has been deemed most cost efficient and informative to be performed at one time, therefore, the selected samples will be analyzed in the coming year.
72 3-D facial images have been captured at the one site where the camera now resides, 40 in children at 6 months of age. Telemedicine protocols were discussed at the site visits to Ukraine in April, 2013 and again in September, 2013. Feasibility of initiating this protocol in Ukraine at this time has been under review. See Dysmorphology Core progress report.

Phase II Continuing Analyses

We currently have one paper in press summarizing the results of the screening of >10,000 women and predictors of risky alcohol use which is we believe the first publication on drinking levels in Ukrainian pregnant women, and demonstrates that the single question on number of drinks a woman can hold in this population accounts for about 30% of the variance in actual reported alcohol consumption in pregnancy, and may provide an efficient approach for identifying high risk women in Ukraine (Chambers et al, 2014).

A second paper on Vitamin D status, demonstrating that recent drinking is associated with lower Vitamin D levels, and that deficiency and insufficiency on this micronutrient is extremely high in this sample, is currently under review (Carlson et al). A third paper led by a post-doctoral fellow in our Division at UCSD demonstrated in PRAMS data from multiple states in the U.S. that use of prenatal/multivitamins is inversely associated with alcohol consumption in the periconceptional period in a dose-response manner (Weiss and Chambers, 2013). Additional publications are in various stages of final analysis of data or completion of discussion sections.

We have completed two additional analyses relative to Phase II. We demonstrated a dose-response relationship with alcohol and physical features and Bayley scores (Table 2), and we demonstrated that two of the additional brain measures on 2D ultrasound (frontothalamic distance and interorbital distance) are significantly predictive of 6 and 12 month Bayley scores (Table 3).

Table 2. Average number of drinks most recent 2 weeks and incidence of features of FASD

Feature	Control N = 256	<3 drinks/wk n = 182	3-7 drinks/wk n = 16	8+ drinks/wk n = 16	Three-group Comparison Alcohol Exposed p-value
PF <10th	20 (7.8%)	**32 (17.7%)**	**6 (37.5%)**	**12 (75.0%)**	**<0.001**
Smooth phil	28 (10.9%)	**21 (11.5%)**	**3 (18.8%)**	**7 (43.8%)**	**0.004**
Thin verm	48 (18.8%)	**36 (19.8%)**	**5 (31.2%)**	**10 (62.5%)**	**<0.001**
OFC <10th	14 (5.5%)	**24 (13.2%)**	**3 (18.8%)**	**7 (43.8%)**	**<0.001**
FAS Dx on features	5 (2.0%)	**8 (4.4%)**	**3 (18.8%)**	**9 (56.2%)**	**<0.001**
6M MDI <85	28/205 (13.7%)	**38/142 (26.8%)**	**7/12 (58.3%)**	**10/13 (76.9%)**	**<0.001**
6M PDI <85	47/204	**46/142**	**6/12**	**9/13**	**0.020**

	(23.0%)	**(32.4%)**	**(50.0%)**	**(69.2%)**	

Table 3. Brain measures on ultrasound in relation to Bayley Scales of Infant Development

Bayley 6 Mos	Ultrasound Measure	N	Alcohol Exposed Group			N	Alcohol Unexposed Group		
			Estimate	SE	p-value		Estimate	SE	p-value
MDI	TCD	105	-0.07	0.65	0.916	172	0.11	0.34	0.746
	OFD	104	0.43	0.23	0.069	171	-0.05	0.14	0.723
	CCD	103	-0.47	0.45	0.300	171	0.40	0.23	0.080
	FTD	**104**	**0.51**	**0.21**	**0.018**	**171**	**0.47**	**0.13**	**<0.001**
	OOD	104	0.47	0.48	0.326	**170**	**0.67**	**0.26**	**0.010**
	IOD	**104**	**-1.22**	**0.46**	**0.010**	**174**	**-1.12**	**0.26**	**<0.001**
	OD	103	1.21	0.85	0.159	171	-0.26	0.52	0.614
PDI	TCD	105	-0.26	0.88	0.770	171	0.32	0.50	0.514
	OFD	104	0.41	0.31	0.186	170	0.04	0.19	0.814
	CCD	103	-0.62	0.60	0.310	170	0.36	0.33	0.274
	FTD	**104**	**0.64**	**0.30**	**0.034**	**172**	**0.67**	**0.17**	**<0.001**
	OOD	104	0.50	0.67	0.457	**169**	**0.75**	**0.37**	**0.043**
	IOD	**104**	**-1.40**	**0.62**	**0.025**	**173**	**-1.43**	**0.35**	**<0.001**
	OD	103	-0.26	1.25	0.835	170	-1.34	0.73	0.070

TCD: transcerebellar diameter; OFD: occipital frontal diameter; CCD: caval calvarial distance; FTD: frontothalamic distance; OOD: outer orbital distance; IOD: inter orbital distance; OD: orbital diameter

Each model adjusted for one or more of the following that met criteria as a confounder: maternal age, parity, socioeconomic status, education, pre—pregnancy BMI, vitamin use and smoking

C. Significance

Implementation of the much more complex protocols in this renewal of the Ukraine cohort study has been time consuming under difficult administrative and political circumstances. Nevertheless, the project continues to progress at the pace planned for most measures, and will be accelerated for the preschool testing battery visits.

The screening paper accepted in ACER has provided a foundation for continued and more widespread concern about prenatal alcohol exposure and has directly led to initiation of a Ministry of Health response. Furthermore, the relationship between the prevalence of risky drinking in this region of the world accompanied by the dose-response incidence data on FASD from our sample support the need for more aggressive interventions. The findings from the ultrasound measures of brain and face are intriguing, and could lead to a clinical application that could be incorporated into routine 2D ultrasound.

D. Plans

1. Accelerate recruitment of children for the 2 and 3.5-5 year old visits in the Spring and Summer months in Rivne and Khmelnytsky to achieve by July, 2014, 90 children in each age group.
2. Perform initial analysis on quality of biological samples from children.
3. Generate preliminary results from Aim 3 and the genetic/epigenetic portion of Aim 4.
4. Provide additional 3D images to Drs. Foroud and Hammond by moving the camera from site to site.
5. Continue with planned and in progress analyses of existing data and publication.

E. Publications

NIH Public Access Compliance	Citation
In process at NIHMS	Chambers CD. Prevalence and Predictors of Maternal Alcohol Consumption in Two Regions of Ukraine. Alcoholism, clinical and experimental research. Forthcoming;
In process at NIHMS	Associations Between Multivitamin Supplement Use and Alcohol Consumption Before Pregnancy: Pregnancy Risk Assessment Monitoring System 2004-2008. Alcoholism, clinical and experimental research.

F. Project Generated Resources

The repository of maternal samples at UCSD is expected to represent a future resource with collaboration of Ukrainian partners. The CIFASD data repository tools developed in this year for data collection on the preschool testing battery are another project generated resource.

Program Director/Principal Investigator (Last, First, Middle): Chambers, Christina

Inclusion Enrollment Report

This report format should NOT be used for data collection from study participants.

Study Title: Earlier Identification of Affected Children and Risk Factors for FASD in Ukraine

Total Enrollment: 179 mothers **Protocol Number:** 080035

Grant Number: 2U01AA014835-10

PART A. TOTAL ENROLLMENT REPORT: Number of Subjects Enrolled to Date (Cumulative) by Ethnicity and Race

Ethnic Category	Females	Males	Sex/Gender Unknown or Not Reported	Total
Hispanic or Latino	0	0	0	0 **
Not Hispanic or Latino	179	0	0	179
Unknown (individuals not reporting ethnicity)	0	0	0	0
Ethnic Category: Total of All Subjects*	179	0	0	179 *
Racial Categories				
American Indian/Alaska Native	0	0	0	0
Asian	0	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	0
Black or African American	0	0	0	0
White	179	0	0	179
More Than One Race	0	0	0	0
Unknown or Not Reported	0	0	**0**	0
Racial Categories: Total of All Subjects*	179	0	0	179 *

PART B. HISPANIC ENROLLMENT REPORT: Number of Hispanics or Latinos Enrolled to Date (Cumulative)

Racial Categories	Females	Males	Sex/Gender Unknown or Not Reported	Total
American Indian or Alaska Native	0	0	0	0
Asian	**0**	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	**0**
Black or African American	0	0	0	0
White	0	0	0	0
More Than One Race	0	0	0	0
Unknown or Not Reported	0	0	0	0
Racial Categories: Total of Hispanics or Latinos**	0	0	0	0 **

* These totals must agree.
** These totals must agree.

Program Director/Principal Investigator (Last, First, Middle): Chambers, Christina

Inclusion Enrollment Report

This report format should NOT be used for data collection from study participants.

Study Title: Earlier Identification of Affected Children and Risk Factors for FASD in Ukraine

Total Enrollment: 42 children **Protocol Number:** 080035

Grant Number: 2U01AA014835-10

PART A. TOTAL ENROLLMENT REPORT: Number of Subjects Enrolled to Date (Cumulative) by Ethnicity and Race

Ethnic Category	Females	Males	Sex/Gender Unknown or Not Reported	Total
Hispanic or Latino	0	0	0	0 **
Not Hispanic or Latino		0	42	42
Unknown (individuals not reporting ethnicity)	0	0	0	0
Ethnic Category: Total of All Subjects*		0	42	42 *
Racial Categories				
American Indian/Alaska Native	0	0	0	0
Asian	0	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	0
Black or African American	0	0	0	0
White		0	42	42
More Than One Race	0	0	0	0
Unknown or Not Reported	0	0	**0**	0
Racial Categories: Total of All Subjects*		0	42	42 *

PART B. HISPANIC ENROLLMENT REPORT: Number of Hispanics or Latinos Enrolled to Date (Cumulative)

Racial Categories	Females	Males	Sex/Gender Unknown or Not Reported	Total
American Indian or Alaska Native	0	0	0	0
Asian	**0**	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	**0**
Black or African American	0	0	0	0
White	0	0	0	0
More Than One Race	0	0	0	0
Unknown or Not Reported	0	0	0	0
Racial Categories: Total of Hispanics or Latinos**	0	0	0	0 **

* These totals must agree.
** These totals must agree.

Targeted/Planned Enrollment Table

This report format should NOT be used for data collection from study participants.

Study Title: Earlier Identification of Affected Children and Risk Factors for FASD in Ukraine

Total Planned Enrollment: 330 children and 450 mothers

TARGETED/PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	**Females**	**Males**	**Total**
Hispanic or Latino	0	0	0
Not Hispanic or Latino	165 (+ 450 mothers)	165	330
Ethnic Category: Total of All Subjects *	165 (+450 mothers)	165	330
Racial Categories			
American Indian/Alaska Native	0	0	0
Asian	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	0	0	0
White	165 (+450 mothers)	165	330
Racial Categories: Total of All Subjects *	165 (+450 mothers)	165	330

* The “Ethnic Category: Total of All Subjects” must be equal to the “Racial Categories: Total of All Subjects.”

PROGRESS REPORT SUMMARY	GRANT NUMBER 2 U01 AA014835-10	
	PERIOD COVERED BY THIS REPORT	
PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR Chambers, Christina	FROM 08/10/2013	THROUGH 05/31/2014

APPLICANT ORGANIZATION
The Regents of the Univ. of Calif., U.C. San Diego

TITLE OF PROJECT (Repeat title shown in Item 1 on first page)
Early Identification of Affected Children and Risk Factors for FASD in Ukraine

A. Human Subjects (Complete Item 6 on the Face Page)		
Involvement of Human Subjects	☒ No Change Since Previous Submission	☐ Change
B. Vertebrate Animals (Complete Item 7 on the Face Page)		
Use of Vertebrate Animals	☒ No Change Since Previous Submission	☐ Change
C. Select Agent Research	☒ No Change Since Previous Submission	☐ Change
D. Multiple PD/PI Leadership Plan	☒ No Change Since Previous Submission	☐ Change
E. Human Embryonic Stem Cell Line(s) Used	☒ No Change Since Previous Submission	☐ Change

SEE PHS 2590 INSTRUCTIONS.

WOMEN AND MINORITY INCLUSION: See PHS 398 Instructions. Use Inclusion Enrollment Report Format Page and, if necessary, Targeted/Planned Enrollment Format Page.

Consistent with the Specific Aims of the application our Activities in support of this contract during the current funding period are as follows:

1. Dr. Coles and Dr. Kable have provided expertise in the development of a preschool battery to assess developmental effects of prenatal alcohol exposure in Ukranian children, ages 3 1/2 to 4 1/2.

2. Drs. Coles and Kable have worked with the Informatics Core and other project personnel on data entry tools for the Ukranian study. Dr. Kable continues to work with the Ukraine Team to train them in using these tools, particularly in relation to the measures that have commercial scoring programs.

4. Dr. Kable has tested a new collection procedure for the attention/physiology protocol on-site in the two sites in the Ukraine. This included trouble shooting on-site when there were problems with instrumentation due to computer differences.

5. Drs. Coles and Kable travelled to Khmelnitsky, Ukraine, April 22-26, 2013, to train staff on administration and scoring of the preschool battery and the attention/physiology procedures.

6. Drs. Coles and Kable travelled to Rivne and Khmelnitsky, Ukraine, September 2013 to evaluate the reliability of test administration and to train staff on scoring preschool protocols.

7. From October 2013 through the present (March 2014), Drs. Coles and Kable have been working remotedly with Ukraine staff to verify scoring procedures and supervise test reliablity using images of test administrations and copies of protocoles. This activity has included regular Skype Conferences.

6. Drs. Coles and Kable have collaborated with Dr. Chambers and other colleagues as required to support actvities of this contract. These activities have included data analysis and preparation of abstracts and presentatons and attending annual CIFASD meetings.

We are not responsible for recruitment and do not work directly with clients. Hence, the Women and Minority question is not relevant to this progress report.

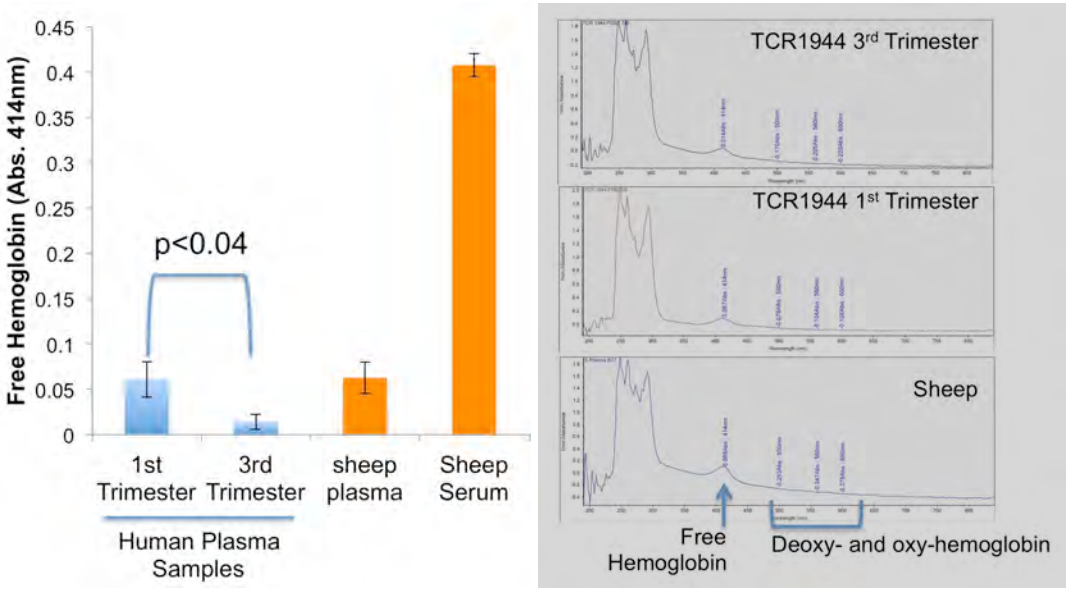
A. Specific Aims: The proposed specific aims are broadly unchanged. We are focused on identifying plasma miRNA biomarkers for maternal alcohol consumption during pregnancy that predict fetal alcohol effect.

B. Studies and Results

During this project period, we accomplished several goals. Firstly, we executed all IRB and biosafety approvals and inter-institutional materials transfer agreements. A total of 142 de-identified patient plasma samples have been transferred to my laboratory at Texas A&M Health Science Center. Each sample was split into two aliquots and stored frozen in two separate -80oC freezers, on two separate backed-up electric circuits to limit the possibility of sample loss. My laboratory is blinded to treatment condition and birth outcomes (these are coded as groups A, B and C), but is not blinded with respect to recruitment site and stage of pregnancy at which the sample was obtained.

Secondly, we developed a set of quality control measures using sheep plasma samples as a test vehicle. Since erythrocytes comprise the largest cell volume in blood, they are an important contaminating source of miRNAs in plasma samples. Moreover, hemolytic anemia can occur in chronic alcoholism as well as later in pregnancy, as part of the HELLP syndrome associated with gestational hypertension and pre-eclampsia. Therefore to assess the contribution of erythrocyte miRNAs to the plasma miRNA profile, we developed a series of quality control steps that we will filter all samples through before adding sample data into the cohort. Firstly, we subject plasma samples to spectrophotometric analysis to quantify free hemoglobin at 414nm as well as whole erythrocyte hemoglobin (500,560 and 600nm). We next assess plasma samples for amplification of a highly expressed erythrocyte mRNA for the Band-3 protein (SLC4A1). Any samples that exhibit SLC4A1 mRNA amplification will be eliminated from analysis. Finally, erythrocytes and leukocytes are highly enriched for specific miRNAs and small nuclear RNAs compared to plasma. We documented this differential expression profile in a sheep model. These data were included in a manuscript accepted for publication in 2014 and form the basis of our quality control measures used for assessing the integrity of human samples. As an additional quality control measure for general cell lysis, we plan to also measure plasma lactate dehydrogenase activity.

Finally, we have now tested two low-density qPCR array platforms from Exiqon and from ABI/Life Technologies. Based on these assessments, we have purchased an initial batch of qPCR arrays from Exiqon as our primary platform. In this initial screen we will be able to assess



A B

Figure 1: (A) Free hemoglobin content in plasma samples obtained from pregnant women in the 1st and 3rd trimesters of pregnancy compared to plasma and serum samples obtained from sheep. (B) Sample absorbance spectrograms of patient plasma from 3rd (top panel) and 1st (middle panel) trimester of pregnancy compared to sheep plasma. The data show that all samples are not contaminated by intact erythrocytes (no detectable peaks for oxy and deoxy hemoglobin) and have minimal contamination with free hemoglobin (414nm). Note that unidentified, plasma specific absorption peaks to the left of the free hemoglobin peak are identical in sheep and human and are not present in erythrocyte samples (data not shown).

miRNA profiles in a total of 60 plasma samples (in the next budget period, we will be able to purchase more qPCR arrays to be able to run additional samples. A set of 10 ABI qPCR arrays is available to validate plasma miRNA expression profiles obtained on the Exiqon platform.

Initial quality assessments of human plasma samples. We performed spectrophotometric assessment on 60 plasma samples divided equally by coded treatment group, stage of pregnancy and recruitment site. None of the samples exhibited whole erythrocyte contamination (erythrocyte bound hemoglobin). Free hemoglobin was within the range attained by sheep plasma samples that we previously found to be depleted for erythrocyte miRNAs. Analysis of variance showed no significant effect of recruitment site ($p<0.62$) or coded treatment ($p<0.39$). There were additionally no interaction effects between recruitment site, coded treatment group or pregnancy stage (all $p<0.46 – 0.63$). However there was a significant main effect of pregnancy stage on free hemoglobin ($p<0.04$, **Figure 1A**). Across all recruitment centers and coded treatment groups, patient plasma samples from the first trimester of pregnancy exhibited on average 4.3-fold higher levels of free hemoglobin compared to samples obtained in the third trimester of pregnancy. However, human plasma samples obtained from the first trimester of pregnancy were not different from sheep plasma samples that were used for miRNA assessment. In contrast, sheep serum-derived samples that are expected to contain substantial amount of hemoglobin exhibited 6.5-fold more hemoglobin than first trimester human plasma samples. Sample absorbance curves for 1st and 3rd trimester human samples and comparison sheep samples are shown in **Figure 1B**.

C. Significance

Since 3rd trimester plasma hemoglobin levels were on average lower than 1st trimester levels, this indicates that late pregnancy associated hypertension and hemolysis is unlikely to be a significant contributory variable to plasma miRNA profiles. Similarly, the lack of coded group differences and differences between recruitment sites indicates that site-specific variables like phlebotomist skill and plasma separation and storage conditions, as well as history of alcohol consumption are unlikely to be significant contributory factors to hemolysis. The first 60 assessed samples pass the first quality control test and will be now assessed for contamination with the additional quality control measure outlined above.

D. Plans

The qPCR arrays represent a significant cost in this project. Therefore, QC measures will be obtained for all samples before the miRNA profiles are assessed. We have purchased reagents to assess 60 samples using the Exiqon platform, and in the next project period, expect to be able to purchase reagents to assess an additional 15 samples. In the next project period, this will enable us to assess miRNA profiles from 1st and 3rd trimester in ~12 patients from each coded group. Plasma miRNA profiles in a random sub-group of 10 of these samples will also be re-assessed using the ABI/Life Technologies platform Data will be processed as outlined in our recently accepted manuscript (ACER 2014, PMID: 24588274, PMCID in progress).

Progress Report Summary

B. Studies and Results

During the period under review, we have finished analysis of all 1077 samples from the 10 shipments for Zn, Cu, Fe, Mg, Ca, transferrin receptor and ceruloplasmin activity. Samples through shipment 9 have been analyzed for homocysteine, C-reactive protein, ferritin and vitamin D. We are in the process of analyzing the remaining samples for choline/choline metabolites, folate and vitamin B12.

D. Plans

We will continue to analyze new maternal and infant plasma samples. Krista Sowell (Ph.D. candidate) has received additional funding from a Campbell Research Award and a Jastro-Shields Award at UCD that will be used to analyze maternal plasma for omega 3/6 fatty acid profiles. She will also be analyzing the maternal plasma samples for vitamins A and E, and select carotenoids. In addition, during the upcoming year, we anticipate writing and submitting 2-3 publications describing the work completed to date.

E. Publications

1. Carlson CR, Uriu-Adams JY, Chambers CD, Yevtushok L, Zymak-Zakutnya N, Chan PH, Wertelecki W and Keen CL. Low vitamin D status in alcohol-exposed and unexposed pregnant Ukrainian women. Alcoholism: Clinical and Experimental Research. Submitted; under review.

A. OBJECTIVES AND SPECIFIC AIMS

There are no changes in the scope or plans for the specific aims of this project. We have continued the acquisition of facial images as well as saliva samples from subjects participating in the CIFASD consortium as well as related projects. We are currently focusing on the collection of a sufficient number of 3D facial images to allow us to perform analyses with the 3DMD camera system. Since our progress report last year, we have collected X new images and X new DNA samples.

Table 1: Summary of data/sample collection through March 1, 2014 (3dMD only)

Site	3D Images (# subjects)[1]	DNA[1]
San Diego	349 (273)	287
UCLA/USC	75 (67)	110
Atlanta	203 (203)	194
Minneapolis	166 (131)	170
Ukraine	47 (34)	0 (no approval)
South Africa (Jacobson)	517 (312)	225[2]
South Africa (May)	37 (37)	0 (no approval)
South Africa (PASS)	2,361 (1221)	0 (collected as part of parent study)
Totals	**3,721 (2,278)**	**986**

[1] Some subjects have now had longitudinal image collection and longitudinal saliva collection (for DNA)

[2] 218 DNA samples also obtained from the mothers and 52 DNA samples obtained from the father. RNA also collected at this site

1) Develop a screening tool that will utilize the data from the 3D facial images and support accurate identification of individuals with a high likelihood of alcohol exposure.

Initial analyses to develop a screening tool focused on the data collected in South Africa in the Cape Coloured population. Over the past year, analyses have been expanded to include the diverse populations recruited in the United States. This has led to some challenges, including the relatively small number of FAS/PFAS subjects in the African American and Hispanic subgroups in the United States. Despite these limitations, consistent results appear to be obtained in the US populations and analyses will continue as the sample sizes in the US expand.

2) Recruit and analyze facial imaging data from very young populations to develop a screening tool that accurately identifies high risk individuals for future intervention.

The accrual of facial images is now complete in the PASS sample in South Africa. A total of 1200 infants were imaged twice, once at 1 month and again at 12 months. Initial analyses were performed in ~250 paired infant images. The PASS investigators have provided identifiers for a set of non-alcohol exposed infants which could be used as controls for these analyses. Using these data, a set of PASS subjects were identified which have a high likelihood of prenatal alcohol exposure. Results of analyses have been presented at the fall PASS meeting. Analyses are now underway in the remaining PASS samples.

Based on the available preliminary results, plans are now underway to expand the imaging of PASS infants to include the US sites as well. Training is anticipated in April with data collection actively

underway during the summer months. The collection of the US samples will provide further diversity and will include a new sample of Native American infants with variable alcohol exposure. Data collection is now underway in the Ukraine and the first set of images has been shipped for analysis.

3) **Combine face images, neurobehavioral data and brain images to identify common pathways and hence improve diagnosis of prenatal alcohol exposure.**

We have recently begun to expand our analyses to include brain imaging data. This is being pursued simultaneously in South Africa, in conjunction with the Jacobson sample, as well as in the US populations. Initial analyses have focused on the paired analysis of the philtrum and the corpus callosum. A much larger set of images (200 per ethnic cohort) is currently being transferred for analysis.

4) **Extend existing and develop novel techniques and associated software to cope with demands of larger datasets and more diverse comparison of controls, alcohol exposed and other developmentally delayed subjects while accommodating multiple anatomical images per subject.**

The migration of the UCL face analysis software from 32 bit to 64 bit is complete. Small "bugs" have arisen occasionally but all have been easily dealt with. Last year's progress report described how a model was constructed for the philtrum patch of 4,747 3D face images of the ALPSAC dataset. We have now been able to extend model construction to the faces of the same image collection and are confident that any limitations on size of dataset we can analyze is now limited only by the RAM available on the computer used. We will continue to test the implementation e.g. with a multi-syndromic model for differential diagnoses of fetal alcohol syndrome and overlapping genetic conditions.

5) **Extend preliminary genetic studies through collection of DNA samples for new subjects and focused analysis to replicate candidate genes identified in basic science components.**

We have continued to collect saliva samples from subjects in the United States. Extracted DNA has been provided to Dipak Sarkar for his pilot study. Analyses have also employed the principal components generated from the analyses performed by Dr. Hammond to test whether there are any genotypic effects on their variation. These analyses have primarily focused to date on candidate genes nominated through various studies.

Publications:

1) McCarthy N, Wetherill L, Lovely CB, Swartz ME, Foroud TM, Eberhart JK. Pdgfra protects against ethanol-induced craniofacial defects in a zebrafish model of FASD. *Development.* 2013 Aug;140(15):3254-65. doi: 10.1242/dev.094938. PMID: 23861062

A. Specific Aims. The aims of this project are unchanged from the proposal.

B. Studies and Results. The current funding year began 6/1/2013 and ends 5/31/2014. Although our initial funding year (2012-2013) was truncated, in this second year of funding we have made considerable progress toward the project goals, which involve standardized neuropsychological assessment at 4 collaborating sites: San Diego State University, Emory University, University of Minnesota, and University of Southern California. Coordination of the project takes place at San Diego State University. During the current year of funding, we focused mainly on data collection, but also continued to address Organization/Administration, Communication, and Training/Reliability. We continued to analyze and publish data from previous funding periods and are starting to examine data from the current phase (CIFASD III). Site Progress Reports are attached.

Organization/Administration. IRB approvals are in place at all sites, and all three subcontracts are fully executed. The SDSU site coordinates purchasing and distributing of materials for all sites. Communication is facilitated by monthly conference calls with site PIs (in addition to the general CIFASD clinical calls).

Training/Reliability. Reliability is an ongoing process: Each site submits every 10th protocol to be reviewed by the SDSU site. New staff are trained either by their own site or by SDSU. All pilot tapes are reviewed by SDSU prior to beginning testing.

Data Collection. As proposed, data collection started on 12/1/12 (in year 1), after training was conducted and reliability established, and is ongoing at all sites. Data collection was hampered slightly by budget restrictions (all sites), staffing issues, and the shortened initial budget year. As of 3/24/14 (16 months of data collection), a total of 301 subjects have been tested across the sites. This represents 94% of our 2-year goal, which is an increase compared to our report of progress last year (65% of 1-year goal). Thus, in terms of the number of subjects tested, we have exceeded our goals for the year. However, all sites have had relatively less success with the younger age group (63% of goal) and the contrast group (61%). All sites are actively trying to remedy this discrepancy. Data collection progress is summarized here, and can also be seen in the enrollment tables.

Site	AE	Control	Contrast	Total Tested	2-year Goal	% of Goal (2nd yr)
SDSU (SMS)						
Old	29	26	15	70	48	146%
Young	8	15	9	32	48	67%
UMN (JWM)						
Old	31	29	17	77	48	160%
Young	11	15	5	31	48	65%
Emory (CCA)						
Old	13	19	6	38	48	79%
Young	15	6	7	28	48	58%
USC (ESL)						
Old	15	10	--	25	32	78%
Subtotal Old	88	84	38	210	176	**119%**
Subtotal Young	34	36	21	91	144	**63%**
Total	122	120	59	301	320	**94%**

Data Management. We continue to work with the informatics team to implement data entry and upload tools for neurobehavioral data from CIFASD III and trouble shoot any developing problems. Data are currently being entered and uploaded into the neurobehavioral and demographics databases. We implemented a tally tool that accurately tracks the number of subjects that have been uploaded to the central repository (CR). This tool counts only subjects that meet inclusion/exclusion criteria for one of our three subject groups and who have complete data (>85%) uploaded to the CR. Only subjects counted by this tally tool are included in the table.

Other Progress. All sites, including the San Diego site, have been collecting MRI data (E. Sowell, PI), genetic material (T. Foroud, PI), 3-D facial photographs (T. Foroud, PI), and dysmorphology data (K. Jones, PI).

Completed Studies. Two papers were published this year and are included in the attached publication list. In addition, we have 2 papers and 2 book chapters under review and several projects in progress, including 2 that were submitted to the upcoming 2014 RSA conference.

1. *Glass et al., Neuropsychological deficits associated with prenatal alcohol exposure are not exacerbated by comorbid ADHD.* This paper was recently published in Neuropsychology.

2. *Glass et al., Correspondence of parent report and laboratory measures of inattention and hyperactivity in children with heavy prenatal alcohol exposure*. This paper is in *Early View* in Neurotoxicology and Teratology.

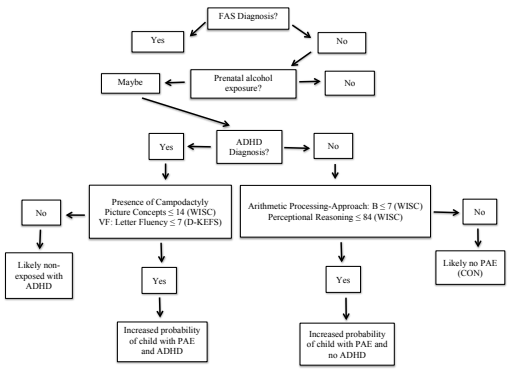
3. *Nguyen et al., The clinical utility and specificity of parent report of executive function among children with prenatal alcohol exposure.* This study, using CIFASD II data, determined that parent reports of executive function can be used to distinguish children with AE +/- ADHD from controls and children with idiopathic ADHD and further demonstrates parent rated exacerbation of behavioral deficits in children with AE and ADHD over children with AE but without ADHD. This paper was submitted for publication and is currently being revised following review.

4. *Glass et al., Effects of age and sex on behavioral and neuropsychological functioning in FASD.* In relation to **Aim 3**, we examined the relation between prenatal alcohol exposure and age on neuropsychological and behavioral performance. Main effects of age indicated that older subjects performed more poorly than younger subjects on both adaptive and cognitive functioning. These effects of age did not differ by group. We also examined the relation between sex and group, but sex was not significant. This study was submitted to RSA.

5. *Adnams et al., Behavior in secondary school learners with prenatal alcohol exposure in South Africa*. Using CIFASD II data, teacher reports of behavior were examined for children with FASD and controls. Groups differed on most domains, even after covariate control. This study was submitted to RSA.

6. *Graham et al*., *Discriminating behavioral subgroups among children with heavy prenatal alcohol exposure.* This study examined heterogeneity in behavior in alcohol-exposed children. Subgroups were characterized by differences in severity rather than type of behavioral problem. Severity was also related to executive function performance. This paper was submitted for publication and is currently being revised following review.

7. *Graham et al., Classification and discrimination analysis*. In keeping with **Aim 1** (to use existing data to develop a tiered or hierarchical approach to identification of affected cases), we attempted classification and regression tree (CART) techniques to analyze data from previous phases. After several different attempts and analytic strategies, we abandoned the CART techniques because they did not allow for manipulation of variables in a way that allowed us to successfully meet our aim. In further analyses, we examined data from 147 Ss with prenatal alcohol exposure and 238 without this exposure. Groups were similar in age, race, handedness and children with FAS were excluded. Both groups included subjects with ADHD. Based on correlational analysis, we selected 19 variables from the neuropsychology, dysmorphology, and 3D facial imaging databases. From these, we determined 3 non-overlapping variables (2 neurobehavioral and 1 dysmorphology) that were successful in discriminating alcohol-exposed subjects with ADHD from non-exposed subjects with ADHD and 2 neurobehavioral variables that were successful in distinguishing alcohol-exposed subjects without ADHD from controls without ADHD (**see figure**). However, our overall classification rates with these variables were not that strong (ranging from 65-96%, but worse for the alcohol-exposed groups) although they were statistically significant. We are considering other analytic strategies, such as discriminant function analysis, in order to build a stronger predictive model.



C. Significance. We have made considerable progress in data collection. As we collect more data, we will be in a better position to meet our study aims (particularly Aims 2-4). Although we have not been successful, thus far, in meeting **Aim 1,** we continue to think it is an important aim and will pursue it further during the next funding year. Our published and ongoing studies add to the literature and increase understanding of the effects of heavy prenatal alcohol exposure on the brain.

D. Plans. During the next funding year we plan to continue data collection, and analyze new and existing data to meet our aims. In particular, we will also be addressing, at each site, the need for additional subjects in the younger age group and in the contrast group, as well as tackling **Aim 1** using different analytic strategies.

E. Publications.

Published Papers: A My NCBI-generated PDF list of publications is included.

Manuscripts Currently Under Review

1. Nguyen, T.T., Glass, L., Coles, C.D., Kable, J.A., May, P.A., Kalberg, W.O., Sowell, E.R., Jones, K.L., Riley, E.P., Mattson, S.N., and the CIFASD. The clinical utility and specificity of parent report of executive function among children with prenatal alcohol exposure.
2. Graham, D.M., Deweese, B.N.,Roesch, S.C., Coles, C.D., Kable, J.A., May, P.A., Kalberg, W.O., Sowell, E.R., Jones, K.L., Riley, E.P., Mattson, S.N., and the CIFASD. Discriminating behavioral subtypes among children with heavy prenatal alcohol exposure.

Book Chapters

1. Glass, L., Ware, A.L. and Mattson, S.N. (accepted, 2013). Neurobehavioral, Neurological, and Neuroimaging Characteristics of Fetal Alcohol Spectrum Disorders. In A. Pffeferbaum and E.V. Sullivan (Eds.), Alcohol and the Nervous System 1E (Handbook of Clinical Neurology). Philadelphia, PA: Elsevier Inc.
2. Graham, D.M., Glass, L., and Mattson, S.N. (submitted, 2014). Teratogen Exposure and Externalizing Behavior. In T.P Beauchaine and S.P. Hinshaw, (Eds.), Oxford Handbook of Externalizing Spectrum Disorders.

Posters Presented During This Funding Period

1. Nguyen, T.T., Glass, L., Coles, C.D., Kable, J.A., May, P.A., Kalberg, W.O., Sowell, E.R., Jones, K.L., Riley, E.P., Mattson, S.N., & the CIFASD (2013). Clinical utility of the Behavior Rating Inventory of Executive Function in the identification of children with prenatal alcohol exposure. Presented at the Research Society on Alcoholism meeting, Orlando, June 2013. Alcoholism: Clinical and Experimental Research, 37, Supplement S2, 44A. DOI: 10.1111/acer.12162, Published online 15 May 2013. http://onlinelibrary.wiley.com/doi/10.1111/acer.12162/abstract

F. Project-Generated Resources.

N/A

Publications Reported for this Reporting Period

NIH Public Access Compliance	Citation
In process at NIHMS	Glass L, Graham DM, Deweese BN, Jones KL, Riley EP, Mattson SN. Correspondence of parent report and laboratory measures of inattention and hyperactivity in children with heavy prenatal alcohol exposure. Neurotoxicol Teratol. 2014 Feb 7;42C:43-50. PubMed PMID: 24512965; NIHMSID: 570774. [Epub ahead of print]
Complete	Glass L, Ware AL, Crocker N, Deweese BN, Coles CD, Kable JA, May PA, Kalberg WO, Sowell ER, Jones KL, Riley EP, Mattson SN, Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD). Neuropsychological deficits associated with heavy prenatal alcohol exposure are not exacerbated by ADHD. Neuropsychology. 2013 Nov;27(6):713-24. PubMed PMID: 24040921; PubMed Central PMCID: PMC3898510.

Program Director/Principal Investigator (Last, First, Middle): Mattson, Sarah N.

Inclusion Enrollment Report

This report format should NOT be used for data collection from study participants.

Study Title: A Multisite Neurobehavioral Assessment of Fetal Alcohol Spectrum Disorders (FASD)

Total Enrollment: 110 **Protocol Number:** SDSU Site

Grant Number: 1 U01 AA014834-11

PART A. TOTAL ENROLLMENT REPORT: Number of Subjects Enrolled to Date (Cumulative) by Ethnicity and Race

Ethnic Category	Females	Males	Sex/Gender Unknown or Not Reported	Total
Hispanic or Latino	18	19	0	37 **
Not Hispanic or Latino	29	43	0	72
Unknown (individuals not reporting ethnicity)	1	0	0	1
Ethnic Category: Total of All Subjects*	48	62	0	110 *
Racial Categories				
American Indian/Alaska Native	1	0	0	1
Asian	1	0	0	1
Native Hawaiian or Other Pacific Islander	1	0	0	1
Black or African American	5	7	0	12
White	30	43	0	73
More Than One Race	10	12	0	22
Unknown or Not Reported	0	0	**0**	0
Racial Categories: Total of All Subjects*	48	62	0	110 *

PART B. HISPANIC ENROLLMENT REPORT: Number of Hispanics or Latinos Enrolled to Date (Cumulative)

Racial Categories	Females	Males	Sex/Gender Unknown or Not Reported	Total
American Indian or Alaska Native	0	0	0	0
Asian	**0**	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	**0**
Black or African American	2	2	0	4
White	10	17	0	27
More Than One Race	6	0	0	6
Unknown or Not Reported	0	0	0	0
Racial Categories: Total of Hispanics or Latinos**	18	19	0	37 **

* These totals must agree.
** These totals must agree.

PROGRESS REPORT SUMMARY	GRANT NUMBER 2 U01 AA014834-10	
	PERIOD COVERED BY THIS REPORT	
PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR Mattson, Sarah	FROM 06/01/2013	THROUGH 05/31/2014

APPLICANT ORGANIZATION
San Diego State University

TITLE OF PROJECT (Repeat title shown in Item 1 on first page)
A Multisite Neurobehavioral Assessment of Fetal Alcohol Spectrum Disorders

A. Human Subjects (Complete Item 6 on the Face Page)		
Involvement of Human Subjects	☒ No Change Since Previous Submission	☐ Change
B. Vertebrate Animals (Complete Item 7 on the Face Page)		
Use of Vertebrate Animals	☒ No Change Since Previous Submission	☐ Change
C. Select Agent Research	☒ No Change Since Previous Submission	☐ Change
D. Multiple PD/PI Leadership Plan	☒ No Change Since Previous Submission	☐ Change
E. Human Embryonic Stem Cell Line(s) Used	☒ No Change Since Previous Submission	☐ Change

SEE PHS 2590 INSTRUCTIONS.

WOMEN AND MINORITY INCLUSION: See PHS 398 Instructions. Use Inclusion Enrollment Report Format Page and, if necessary, Targeted/Planned Enrollment Format Page.

Marcus Subcontract Progress Report as of March 7, 2014
In this last year, we have done the following in accordance with the Aims of the project:

1) We have enrolled 72 participants into the CIFASD-III protocol to date. Data from these participants were encoded, verified, and uploaded to the central repository.
2) Dr. Jones completed a physical exam on 55 participants and 3-D imaging of the participants was also conducted by Leah Wetherill. Participants were those enrolled in CIFASD-2 and CIFASD-3 protocols in 2012-2013.
3)JiSoo Park and Julia Ouellette were hired as a work study to assist with data entry for the project in the 2013-14 year.
4) Additional staff was hired to replace Chelsea Oswald who decided to leave the project after the birth of her daughter in December of 2013 to enable her to have family leave prior to transitioning to her clinical internship in the Summer of 2014. In addition, Shital Gaitonde, a former tester, had to be replaced. She continued testing until March of 2014 but discontinued her involvement with the project to devote more time to her private practice. Sophie Green, Allie Ramsey, and Brandi Smith were added to the staff as master level psychometrists.
5) Sophie Green was hired in November and completed her piloting testing in early February. She is actively seeing participants.
6) Allie Ramsey and Brandi Smith were hired in December and have submitted their pilot protocols for approval. We anticipate that both will be able to assist the project with testing later in March of 2014.
7)Additional data encoding and uploading to the central repository was done on data collected from 2012-2013 for the CIFASD-II protocol.
8) Sharon Paige Whitaker, our recruitment specialist, has networked with other recruitment staff in the Department of Psychiatry at EUSM to expand our recruitment of the clinical contrast group. She attends monthly meetings where information is shared on study protocols and has prepared materials to be included in an expanded reseach protocol book available to patients who come into the Emory Healthcare service.

Marcus Subcontract Progress Report as of March 7, 2014

6)Reviewed manuscripts from the CIFASD II project as requested.

Plans
1) Recruit additional participants to meet yearly goals of CIFASD-3
2) Complete the physical examination and 3-D imaging on the participants seen in the last year. An anticipated 50-60 participants will be seen by Dr. Jones and photographed by Jeff Rogers on April 25-26.
3) Our facilitiates are being relocated to another building in the Emory area in March/April of 2014 so staff will have to assist with the transition and recruitment materials will have to be modified to inform participants of the location change.
4) Attend community health fairs and events to assist with recruiting typically developing children in the metro-Atlanta area.
5) Encode, verify, and upload new data into the central repository.
6) Attend the annual CIFASD meeting in April of 2014.

Program Director/Principal Investigator (Last, First, Middle): Mattson, Sarah N. (Emory subaward- Kable)

Inclusion Enrollment Report

This report format should NOT be used for data collection from study participants.

Study Title: A Multisite Neurobehavioral Assessment of Fetal Alcohol Spectrum Disorders: EUSM

Total Enrollment: 13 **Protocol Number:** IRB00061788

Grant Number: 2 U01 AA014834-11

PART A. TOTAL ENROLLMENT REPORT: Number of Subjects Enrolled to Date (Cumulative) by Ethnicity and Race

Ethnic Category	Females	Males	Sex/Gender Unknown or Not Reported	Total
Hispanic or Latino	0	6	0	6 **
Not Hispanic or Latino	34	33	0	67
Unknown (individuals not reporting ethnicity)	0	0	0	0
Ethnic Category: Total of All Subjects*	34	39	0	73 *
Racial Categories				
American Indian/Alaska Native	0	0	0	0
Asian	0	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	0
Black or African American	25	29	0	54
White	6	10	0	16
More Than One Race	3	0	0	3
Unknown or Not Reported	0	0	**0**	0
Racial Categories: Total of All Subjects*	34	39	0	73 *

PART B. HISPANIC ENROLLMENT REPORT: Number of Hispanics or Latinos Enrolled to Date (Cumulative)

Racial Categories	Females	Males	Sex/Gender Unknown or Not Reported	Total
American Indian or Alaska Native	0	0	0	0
Asian	**0**	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	**0**
Black or African American	0	0	0	0
White	0	6	0	6
More Than One Race	0	0	0	0
Unknown or Not Reported	0	0	0	0
Racial Categories: Total of Hispanics or Latinos**	0	6	0	6 **

* These totals must agree.
** These totals must agree.

PROGRESS REPORT SUMMARY	GRANT NUMBER 5 U01 AA014834-10	
	PERIOD COVERED BY THIS REPORT	
PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR Elizabeth R. Sowell	FROM 06/01/2013	THROUGH 05/31/2014

APPLICANT ORGANIZATION
Children's Hospital Los Angeles

TITLE OF PROJECT (Repeat title shown in Item 1 on first page)
A Multisite Neurobehavioral Assessment of Fetal Alcohol Spectrums Disorders

A. Human Subjects (Complete Item 6 on the Face Page)		
Involvement of Human Subjects	☒ No Change Since Previous Submission	☐ Change
B. Vertebrate Animals (Complete Item 7 on the Face Page)		
Use of Vertebrate Animals	☒ No Change Since Previous Submission	☐ Change
C. Select Agent Research	☒ No Change Since Previous Submission	☐ Change
D. Multiple PD/PI Leadership Plan	☒ No Change Since Previous Submission	☐ Change
E. Human Embryonic Stem Cell Line(s) Used	☒ No Change Since Previous Submission	☐ Change

SEE PHS 2590 INSTRUCTIONS.

WOMEN AND MINORITY INCLUSION: See PHS 398 Instructions. Use Inclusion Enrollment Report Format Page and, if necessary, Targeted/Planned Enrollment Format Page.

Since study initiation, we have completed 15 control subjects and 23 alcohol exposed subjects on neuropsychological testing (as of 3/12/14). Two participants are scheduled in the near future. Our new CIFASD project coordinator, Trinh Luu began on 02/03/2014. A new CIFASD research assistant, Alexy Andrade is currently training on the CIFASD III neuropsychological testing battery and will be piloting for approval on 3/20/14. Race, ethnicity and gender details are available on the inclusion enrollment report. We are currently meeting our projected recruitment goals.

Program Director/Principal Investigator (Last, First, Middle): Mattson, Sarah N. (Subaward CHLA-Sowell)

Inclusion Enrollment Report

This report format should NOT be used for data collection from study participants.

Study Title: A Multisite Neurobehavioral Assessment of Fetal Alcohol Spectrum Disorders

Total Enrollment: 38 **Protocol Number:**

Grant Number: 5 U01 AA014834-10

PART A. TOTAL ENROLLMENT REPORT: Number of Subjects Enrolled to Date (Cumulative) by Ethnicity and Race

Ethnic Category	Females	Males	Sex/Gender Unknown or Not Reported	Total
Hispanic or Latino	1	09		10 **
Not Hispanic or Latino	13	13		26
Unknown (individuals not reporting ethnicity)		2		2
Ethnic Category: Total of All Subjects*	14	24		38 *
Racial Categories				
American Indian/Alaska Native		1		1
Asian	1	1		2
Native Hawaiian or Other Pacific Islander	0	0		0
Black or African American	3	1		4
White	7	11		18
More Than One Race	3	8		11
Unknown or Not Reported		2		2
Racial Categories: Total of All Subjects*	14	24		38 *

PART B. HISPANIC ENROLLMENT REPORT: Number of Hispanics or Latinos Enrolled to Date (Cumulative)

Racial Categories	Females	Males	Sex/Gender Unknown or Not Reported	Total
American Indian or Alaska Native		1		1
Asian				
Native Hawaiian or Other Pacific Islander				
Black or African American				
White	1	3		4
More Than One Race		3		3
Unknown or Not Reported		2		2
Racial Categories: Total of Hispanics or Latinos**	1	9		10 **

* These totals must agree.
** These totals must agree.

PROGRESS REPORT SUMMARY	GRANT NUMBER U01AA014834-10	
	PERIOD COVERED BY THIS REPORT	
PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR Jeffrey R. Wozniak, Ph.D.	FROM 06/10/2013	THROUGH 05/31/2014

APPLICANT ORGANIZATION
Regents of the University of Minnesota

TITLE OF PROJECT (Repeat title shown in Item 1 on first page)
A multi-site neurobehavioral assessment of fetal alcohol spectrum disorders

A. Human Subjects (Complete Item 6 on the Face Page)		
Involvement of Human Subjects	☒ No Change Since Previous Submission	☐ Change
B. Vertebrate Animals (Complete Item 7 on the Face Page)		
Use of Vertebrate Animals	☒ No Change Since Previous Submission	☐ Change
C. Select Agent Research	☒ No Change Since Previous Submission	☐ Change
D. Multiple PD/PI Leadership Plan	☒ No Change Since Previous Submission	☐ Change
E. Human Embryonic Stem Cell Line(s) Used	☒ No Change Since Previous Submission	☐ Change

SEE PHS 2590 INSTRUCTIONS.

WOMEN AND MINORITY INCLUSION: See PHS 398 Instructions. Use Inclusion Enrollment Report Format Page and, if necessary, Targeted/Planned Enrollment Format Page.

A. Specific Aims: There are no changes to the aims for this project from those stated in the initial application.

B. Studies and Results: Data collection is underway and is proceeding on target. No results are available yet as this is only the first year of this project.

C. Significance: This work is highly significant to families of children impacted by Fetal Alcohol Spectrum Disorder. CIFASD represents the largest collection of clinical, neurobehavioral, MRI, and genetic data from children with Fetal Alcohol Spectrum Disorders at present. The large pool or shared data and expertise of investigators at multiple sites will allow for significant new insights into the neurodevelopmental consequences of prenatal alcohol exposure.

D. Plans: Over the next year of the project, we plan to enroll additional participants (FASD, controls, and clinical contrast participants) in the CIFASD neurobehavioral project and to share these data with the consortium.

Human Subjects: The project is current with our IRB (#0306M48644).

E. Publications: None to date.

F. Project-Generated Resources: NA

Program Director/Principal Investigator (Last, First, Middle): Mattson, Sarah N. (Minnesota subaward- Wozniak)

Inclusion Enrollment Report

This report format should NOT be used for data collection from study participants.

Study Title: A multi-site neurobehavioral assessment of fetal alcohol spectrum disorders

Total Enrollment: 109 **Protocol Number:** 0306M48644

Grant Number: U01AA014834-11

PART A. TOTAL ENROLLMENT REPORT: Number of Subjects Enrolled to Date (Cumulative) by Ethnicity and Race

Ethnic Category	Females	Males	Sex/Gender Unknown or Not Reported	Total
Hispanic or Latino	4	3	0	7 **
Not Hispanic or Latino	45	56	0	101
Unknown (individuals not reporting ethnicity)	0	1	0	1
Ethnic Category: Total of All Subjects*	49	60	0	109 *
Racial Categories				
American Indian/Alaska Native	2	1	0	3
Asian	1	2	0	3
Native Hawaiian or Other Pacific Islander	0	0	0	0
Black or African American	10	4	0	14
White	28	43	0	71
More Than One Race	8	9	0	17
Unknown or Not Reported	0	1	**0**	1
Racial Categories: Total of All Subjects*	49	60	0	109 *

PART B. HISPANIC ENROLLMENT REPORT: Number of Hispanics or Latinos Enrolled to Date (Cumulative)

Racial Categories	Females	Males	Sex/Gender Unknown or Not Reported	Total
American Indian or Alaska Native	0	0	0	0
Asian	**0**	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0	**0**
Black or African American	0	0	0	0
White	4	3	0	7
More Than One Race	0	0	0	0
Unknown or Not Reported	0	0	0	0
Racial Categories: Total of Hispanics or Latinos**	4	3	0	7 **

* These totals must agree.
** These totals must agree.

A. SPECIFIC AIMS

The Specific Aims are unchanged from the original application.

B. STUDIES AND RESULTS

Important progress has been made in the last year in achieving the aims of the imaging core of the Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD), 5U01AA017122. Though data acquisition has been a focus during this phase of the project, progress regarding methods development and new image analysis has been conducted and achieved in tandem. Our group continues to work on data collected from CIFASD II, and, in the last year, we have submitted 2 peer review manuscripts from longitudinal data, one provisionally accepted in *Cerebral Cortex* (impact factor 6.8), and another in revision at *Neuroimage: Clinical.* We have 3 other manuscripts in preparation, and have presented these data at numerous conferences in the last year. We describe some of these findings in the Preliminary Studies section below. CIFASD III time 1 brain imaging data collection is near completion (with 174 participants imaged), and most of the subjects have all aspects of CIFASD III projects, including neurocognitive, dysmorphology, and 3D facial imaging data. We are in the process of developing integrative analyses on cross-sectional aspects of the study.

Brain Image Acquisition:

CHLA: We have had significant personnel changes in the project coordinator position at our site, and were without a project coordinator for approximately 4 months in the last year. We are pleased to share that we have found an extremely well qualified CIFASD project coordinator, Trinh Luu, who has a masters degree in psychology, and significant experience in brain imaging, IRB, subject recruitment, and more from her work in a brain imaging lab at UCLA. Despite the turn-over, we have collected data and the neurocognitive battery on 39 participants (24 exposed, 15 controls), and have numerous recruits coming in the next few months.

SDSU: Funding of this award began 8/1/2012 and this subcontract has been in place since 11/9/2012. During the current funding year, we have continued to work with the PI and data collection is continued. To date, we have scanned 23 alcohol-exposed and 12 control subjects. We are aiming for 30 subjects in each group for time 1 data collection. The difference is related to the considerable difficulties in our initial data collection procedures, which were described previously, as well as the shortened funding year in year 06. Of the 108 subjects that have been tested neurobehaviorally (thru 3/7/2014) as part of Dr. Mattson's U01 project, 101 were screened, and 48 were scan eligible (excluded are children out of the age range, those in the "contrast" group, and those who have MRI contraindications). Of the 48 eligible, 2 have declined MRI scanning. We have conducted mock scans (to ease anxiety and increase likelihood of collecting quality data on the scan day) and actual scans of 39 subjects (13 CON and 26 AE). 2 subjects are currently scheduled for testing.

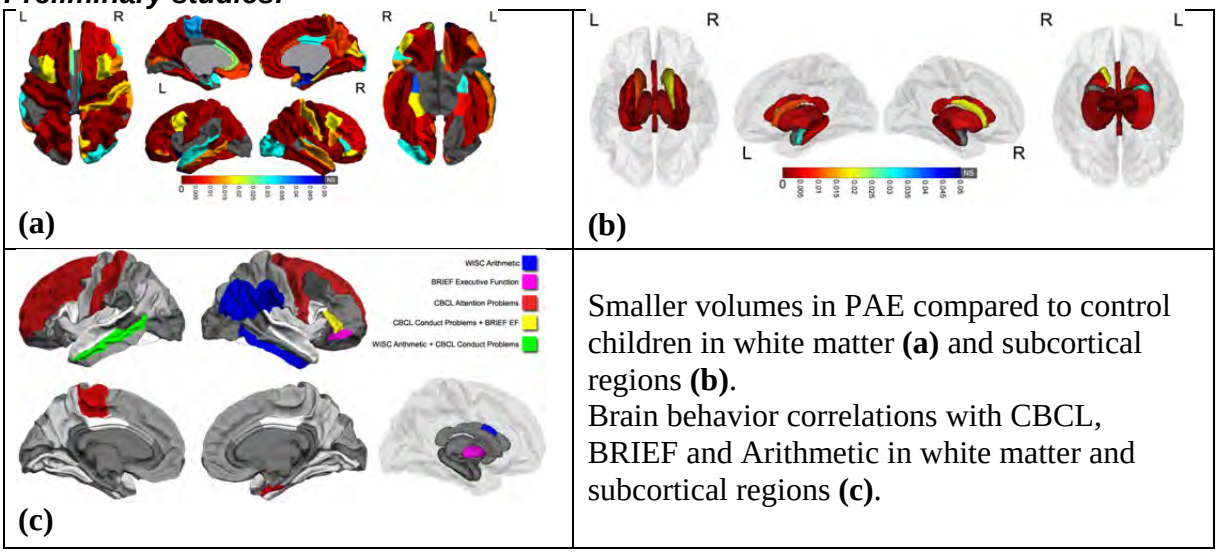
Emory: In support of the Imaging study being carried out by Dr. Sowell and her colleagues, Emory will recruit 48 school aged children (7 to 17 years) in two categories: 1) those with a verifiable history of prenatal alcohol exposure or FASD and 2) a control group of unexposed children. These children will have completed a neurobehavioral battery as part of Dr. Mattson Neurodevelopmental Core and will be evaluated by Dr. Jones as part of the Dysmorphology Core. These children will be imaged by staff at the Biomedical Imaging Technology Center (BITC) at Emory University (Xiaoping Hu, PhD, Director). Subsequently, in the 2nd two years of the contract, these children will be reimaged using the same protocol. In the first two years of the contract, there are 21 months available for recruitment due to the shortening of the contract period in Year I. As of 3/5/14, 38 of the 48 children have been recruited and scanned. All children are part of the larger CIFASD project and, have been tested using the Neurocognitive Battery. Later this month, all of these children will be evaluated by Ken Jones, will have saliva samples for DNA collected, and will have 3D facial imaging.

UMN: Data collection is well underway. At the time of preparation of this report, a total of 59 scans have been completed. This work is highly significant to families of children impacted by Fetal Alcohol Spectrum Disorder. CIFASD represents the largest collection of clinical, neurobehavioral, MRI, and genetic data from children with Fetal Alcohol Spectrum Disorders at present. The large pool or shared data and expertise of investigators at multiple sites will allow for significant new insights into the neurodevelopmental consequences of prenatal alcohol exposure. Over the next year of the project, we plan to enroll and scan the remainder of our allotted participants in the CIFASD neurobehavioral project and to share these data with the consortium (at the time of preparation of this report. Screening of the already-collected data will tell us whether we have participants who need to be “replaced” because of bad MRI data.

Data Usability: Of the 174 participants imaged at the time of this report, only 4 T1-weighted images are “unusable” for image analysis purposes due to extreme motion artifact, and another 14 are of questionable quality for use in analyses. Thus, 90% of data collected can definitely be used for proposed analyses, and

another 6% are likely to be at least partially usable. While more work on data quality is in process for the DTI and resting-state fMRI data, good T1 data is most often consistent with the other imaging modalities.

Preliminary studies:



Smaller volumes in PAE compared to control children in white matter **(a)** and subcortical regions **(b)**.
Brain behavior correlations with CBCL, BRIEF and Arithmetic in white matter and subcortical regions **(c)**.

During the current budget cycle, image analysis led by Dr. Sowell's group has incorporated longitudinal examination of changes in functional and structural brain characteristics to determine the continued impact of prenatal exposure to alcohol on the developing brain. For examinations of brain activation, results have revealed significant effects of prenatal alcohol on working memory and attention during brain maturation. Further, the trajectories of task-related brain activity distinguish exposed and non-exposed individuals across development (Gautam et al., submitted). In an independent study, significant effects of prenatal alcohol exposure on age-related changes in white matter volume and executive function were also detected (Gautam et al., submitted). These results support that the harmful effects of prenatal alcohol extend well beyond the initial insult and lead to altered neuroplasticity during critical stages of brain maturation. During this funding period, we have also continued to develop innovative techniques for identifying relationships between brain imaging measures with neurobehavioral deficits and facial characteristics in cross sectional analyses. With respect to white matter development, data was processed through the longitudinal stream in FreeSurfer v5.1, and volumes were extracted for 82 white matter and subcortical regions (41/hemisphere) and 5 callosal areas. Preliminary analysis tested group volume differences and used false discovery rate (FDR) correction. For regions showing significant group effects, mixed models examined group-age interactions and correlations between change in volume and raw WISC arithmetic, CBCL, and BRIEF scores. Most regions showed significant (FDR-corrected) differences in volume across group (Fig. 1 **a, b**). Group interactions were significant for WISC arithmetic scores in 6 regions, CBCL attention scores in 8 regions, CBCL conduct scores in 2 regions, and BRIEF executive function scores in 4 regions **(Fig. 1 c).** In PAE, poorer cognitive or behavioral outcomes were significantly related to white matter volume increases in most regions that showed group interactions. These associations were largely absent in control subjects. Volumetric differences appeared consistent across childhood in the PAE and control groups, suggesting similar trajectories of subcortical volume development. We have also refined techniques that allow for the characterization of relationships between cortical gyrification, which begins around mid-gestation in humans, prenatal alcohol exposure and anthropomorphic measures of facial dysmorphology. Results point to relatively pervasive reductions in the complexity of gyrification patterns that encompass prefrontal, parietal and inferior temporal association regions in children exposed to alcohol prenatally compared to non-exposed controls. Relationships between regional gyrification indices and dysmorphology measures and IQ are further observed suggesting a common pathophysiology. Results from this study were presented as a platform presentation at the 19th Annual Meeting of the Organization for Human Brain Mapping in June of this year and are poised for submission in manuscript format (Joshi et al., in preparation).

C. SIGNIFICANCE (FROM ORIGINAL APPLICATION)

Understanding the neurobiological consequences of prenatal alcohol exposure is a fundamental first step for determining when and how to best intervene to improve cognitive and functional outcomes. While prenatal alcohol exposure is the leading preventable cause of intellectual disabilities, the prevalence of fetal alcohol syndrome is not declining, and the prevalence of those negatively affected by prenatal alcohol exposure, but without the defining facial dysmorphology to warrant the full FAS diagnosis is likely much higher. Thus, while prevention is key, developing intervention strategies is still a priority, and the brain imaging investigations proposed here can help drive these efforts forward.

E. PUBLICATIONS

Articles under review

1. Gautam P, Nuñez SC, Narr KL, Mattson SN, May PA, Adnams CM, Riley EP, Jones KL, Kan EC, and Sowell ER. Prenatal exposure to alcohol alters developmental trajectories for visuo-spatial attention: multisite longitudinal fMRI study. *Cerebral Cortex*, 2014; provisionally accepted.
2. Gautam P, Nuñez SC, Narr KL, Kan E, and Sowell ER. Effects of prenatal alcohol exposure on the development of white matter volume and executive function: A longitudinal MRI study. N*euroImage: Clinical*, 2014; under revision.

Articles in preparation

1. Uban KA, Lynch KM, Herting MM, Gautam P, Nunez SC, Colby JB, Kan EC, Adnams CM, May PA, Narr KL, Mattson SN, Riley EP, and Sowell ER. Associations between structural connectivity and cognition in youth prenatally exposed to alcohol. In preparation, 2014.
2. Gautam P,Lebel C, Narr KL, Mattson SN, May PA, Adnams CM, Riley EP, Jones KL, Kan EC, Sowell ER. Volume changes and brain-behavior relationships in subcortical white and gray matter in children with prenatal alcohol exposure. In preparation, 2014.
3. Gautam P, Nuñez SC, Narr KL, Mattson SN, May PA, Adnams CM, Riley EP, Jones KL, Kan EC, Sowell ER. Change in white matter connectivity and executive function in fetal alcohol spectrum disorders. In preparation, 2014.
4. S. H. Joshi, K. L. Narr, E. Kan, R. P. Woods, A. W. Toga, S.N. Mattson, E.P. Riley, K.L. Jones, C.M. Adnams, P.A. May, M.J. O'Connor, and E. Sowell. Abnormal Patterns of Gyrification in Fetal Alcohol Syndrome. In preparation, 2014.

Abstract Presentations

1. Uban KA, Herting MM, & Sowell ER. Sexually dimorphic alterations in structural connectivity in youth with a fetal alcohol spectrum disorder. The 36th Annual Research Society on Alcoholism Scientific Meeting, June 2013.
2. Gautam P, Nunez SC, Kan E, Riley ED, Mattson S, Sowell ER. Longitudinal changes in white matter in children prenatally exposed to alcohol: Comparisons with typical controls. The 19th Annual Meeting of the Organization for Human Brain Mapping, Seattle, WA June 2013
3. Uban KA, Herting MM, Colby JB, Gautam P & Sowell ER. Track base spatial statistic reveal alterations in structural connectivity in male and female adolescents with a fetal alcohol spectrum disorder. The Inaugural Flux Congress, Pittsburgh, PA, Sept. 2013
4. Gautam P, Nuñez SC, Mattson S, Riley ED, Herting MM, Uban KA, Colby J, Kan E, Sowell ER. Change in cognitive function is associated with connectivity changes in fetal alcohol spectrum disorders. The Inaugural Flux Congress conference, Pittsburgh, PA, Sept. 2013.
5. Gautam P, Nunez SC, Riley ED, Mattson S, Herting MM, Uban KA, Colby J, Kan E, Sowell ER. Longitudinal change in cognitive function is associated with structural connectivity changes in prenatally exposed to alcohol. The 43rd Society for Neuroscience Annual Meeting, San Diego, CA, Nov. 2013.
6. Gautam P, Narr KL, Mattson SN, May PA, Adnams CM, Riley EP, Jones KL, Kan EC, Sowell ER, Lebel C. (Sub)cortical volume changes and brain-behavior relationships in youth prenatally exposed to alcohol. The 20th Annual Meeting of the Organization for Human Brain Mapping, Hamburg, Germany, June 2014.
7. Uban KA, Lynch KM, Andrade AF, Herting MM, Gautam P, Nuñez SC, Colby JB, Kan EC, Adnams C, May P, Narr K, Mattson S, Riley E, Sowell ER. Associations between structural connectivity and cognition in youth prenatally exposed to alcohol. The 20th Annual Meeting of the Organization for Human Brain Mapping, Hamburg, Germany, June 2014.
8. Lynch KM, Uban KA, Herting MM, Gautam P, Colby JB, Kan EC, Adnams C, May P, Narr K, Sowell ER. Prenatal Alcohol Exposure Associations with Structural Connectivity Differ in Boys and Girls. The 20th Annual Meeting of the Organization for Human Brain Mapping, Hamburg, Germany, June 2014.
9. Uban K, Lynch K, Herting M, Gautam P, Kan EC, & Sowell ER. Along-tract statistic reveal alterations in structural connectivity in male and female adolescents with a fetal alcohol spectrum disorder. The 37th annual Research Society on Alcoholism Scientific Meeting, Bellevue, WA, June 2014.

Program Director/Principal Investigator (Last, First, Middle): Sowell, Elizabeth R.

Inclusion Enrollment Report

This report format should NOT be used for data collection from study participants.

Study Title: Mapping the Brain, the Face and Neurocognitive Function in FASD (U01)

Total Enrollment: 178 **Protocol Number:**

Grant Number: 5 U01 AA017122-07

PART A. TOTAL ENROLLMENT REPORT: Number of Subjects Enrolled to Date (Cumulative) by Ethnicity and Race

Ethnic Category	Females	Males	Sex/Gender Unknown or Not Reported	Total
Hispanic or Latino	10	18		28 **
Not Hispanic or Latino	66	81		147
Unknown (individuals not reporting ethnicity)	1	2		3
Ethnic Category: Total of All Subjects*	77	101		178 *
Racial Categories				
American Indian/Alaska Native	3	2		5
Asian	3	3		6
Native Hawaiian or Other Pacific Islander	0	0		0
Black or African American	26	16		42
White	29	60		89
More Than One Race	16	18		34
Unknown or Not Reported		2		2
Racial Categories: Total of All Subjects*	77	101		178 *

PART B. HISPANIC ENROLLMENT REPORT: Number of Hispanics or Latinos Enrolled to Date (Cumulative)

Racial Categories	Females	Males	Sex/Gender Unknown or Not Reported	Total
American Indian or Alaska Native		1		1
Asian				
Native Hawaiian or Other Pacific Islander				
Black or African American	1	1		2
White	7	11		18
More Than One Race	2	3		5
Unknown or Not Reported		2		2
Racial Categories: Total of Hispanics or Latinos**	10	18		28 **

* These totals must agree.
** These totals must agree.

PROGRESS REPORT SUMMARY	GRANT NUMBER AA021651-03	
	PERIOD COVERED BY THIS REPORT	
PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR Sulik, Kathleen K..	FROM 08/10/2013	THROUGH 05/31/2014

APPLICANT ORGANIZATION
University of North Carolina at Chapel Hill

TITLE OF PROJECT (Repeat title shown in Item 1 on first page)
Craniofacial and CNS pathology in a mouse FASD model

A. Human Subjects (Complete Item 6 on the Face Page)		
Involvement of Human Subjects	☒ No Change Since Previous Submission	☒ Change
B. Vertebrate Animals (Complete Item 7 on the Face Page)		
Use of Vertebrate Animals	☒ No Change Since Previous Submission	☐ Change
C. Select Agent Research	☒ No Change Since Previous Submission	☐ Change
D. Multiple PD/PI Leadership Plan	☒ No Change Since Previous Submission	☐ Change
E. Human Embryonic Stem Cell Line(s) Used	☒ No Change Since Previous Submission	☐ Change

SEE PHS 2590 INSTRUCTIONS.

WOMEN AND MINORITY INCLUSION: See PHS 398 Instructions. Use Inclusion Enrollment Report Format Page and, if necessary, Targeted/Planned Enrollment Format Page.

Objectives: The primary objective of this work is to make clinically significant discoveries regarding prenatal alcohol (ethanol) exposure-induced pathology involving the brain and face. This work naturally builds on our CIFASD-supported basic research to date and continues to address the need for a more complete understanding of the spectrum and exposure stage-dependency of abnormalities caused by prenatal alcohol exposure. Our overall hypothesis is that alcohol induces structural abnormalities of the brain and face of mice that are consistent with and informative for those in human FASD. The 3 original Specific Aims for this grant are as follows: Aim 1. to examine correlations between the dysmorphology of the brain and the face that result from alcohol exposure at specific early stages of prenatal development in mice; Aim 2. to delineate early prenatal alcohol exposure-induced cerebral cortical thickness alterations and associated fiber tract and structural connectivity changes in postnatal mice; and Aim 3. to further define the histopathology and genesis of early prenatal alcohol exposure-induced regional brain dysmorphology.

Methods: Methods: Correlative brain and face dysmorphology studies are being conducted on fetal mice using high-resolution Magnetic Resonance Imaging (MRI) and dense surface modeling (DSM). Fetal animals are being collected from groups of control dams as well as from those that had been administered alcohol acutely on days 10 or 11.5 of pregnancy. Additionally, the brains of postnatal mice whose dams had been treated acutely on day 7 of pregnancy are being examined using atlas-based MRI & DTI analysis techniques to assess regional brain volumes, cortical thickness changes, and fiber tract alterations. Magnesium enhanced MRI (MEMRI) techniques are being developed and applied to enhance our understanding of structural/functional relationships. Histological methodologies are also being employed for analysis of regions of interest in pre- and postnatal brains. In addition to these structural analysis techniques, mutant mice are being used to examine gene/environment interactions; a high potency cannabinoid, CP-55940, is being used for a co-teratogen exposure study; and a sonic hedgehog (Shh) pathway agonist (SAG) is being employed to further explore the role of modulated Shh signaling in the genesis of alcohol-induced abnormalities.

Accomplishments and Results: Overall, our progress during this funding period (6/01/2013-5/31/14) has been steady and substantial. During this time our CIFASD-funded publications have included four published papers and an additional one that is in press, along with 9 abstracts. Among the presentations made during this reporting period was an invited talk by Dr. Sulik regarding her lab's CIFASD-supported research findings for an FASD legal issues conference in Edmonton, Alberta Canada. Importantly, during this time period, Dr. Shonagh O'Leary-Moore left academia and Dr. Rob Lipinski took a faculty position at the University of Wisconsin. In adjusting to this change in our lab personnel, and in order to continue to productively address our primary research objective, Dr. Eric Fish (research associate), Dr. Lindsay Wieczorek (postdoctoral fellow), and Dr. Scott Parnell (assistant Professor) have joined our CIFASD effort. Dr. Lipinski remains a collaborator, as do Dr. Al Johnson, Duke University and Dr. Peter Hammond and Mike Suttie, University College of London.

During this period, MRI scanning for stage of exposure-dependent brain and facial dysmorphology analyses of GD 17 fetuses has been successfully completed for 6 litters that were exposed to ethanol on GD10, for 5 litters that were exposed to ethanol on GD11.5, and for 7 control litters. The data from these scans is currently being registered and processed through the pipeline. Although in the past our work has relied heavily on manual segmentation of fetal brains, we have been working with Dr. Hammond and Mike Suttie to apply atlas-based regional brain segmentations for brain shape and volume analyses. This provides for a substantial reduction in image processing time and in consequent financial savings.

Following up on our previous study employing MRI and Dense Surface Modeling to study and compare brain and facial dysmorphology resulting from GD7 or 8.5 alcohol exposure, and in keeping with the consortium's interest in genetically-based susceptibilities (gene/environment interactions), we have conducted and reported a study illustrating that Shh and Gli2 mutation in mice greatly increases GD7 alcohol exposure-induced teratogenesis (Kietzman et al., 2014, Figure 1). Additionally, we have initiated a similar GD8.5 study, testing the hypothesis that developmental stage-dependent alterations in the Shh signaling pathway can yield opposing phenotypes; i.e. a face and brain that are too narrow from GD7 alcohol exposure versus too wide from GD 8.5 alcohol exposure. To further illustrate that insult to the Shh pathway underlies alcohol-induced teratogenesis, in collaboration with Dr. Kevin Williams at North Carolina Central University, a study employing the Shh pathway (Smoothened) agonist, SAG, has been initiated. This study is designed to test the hypothesis that by upregulating Smoothened, alcohol teratogenesis can be modified.

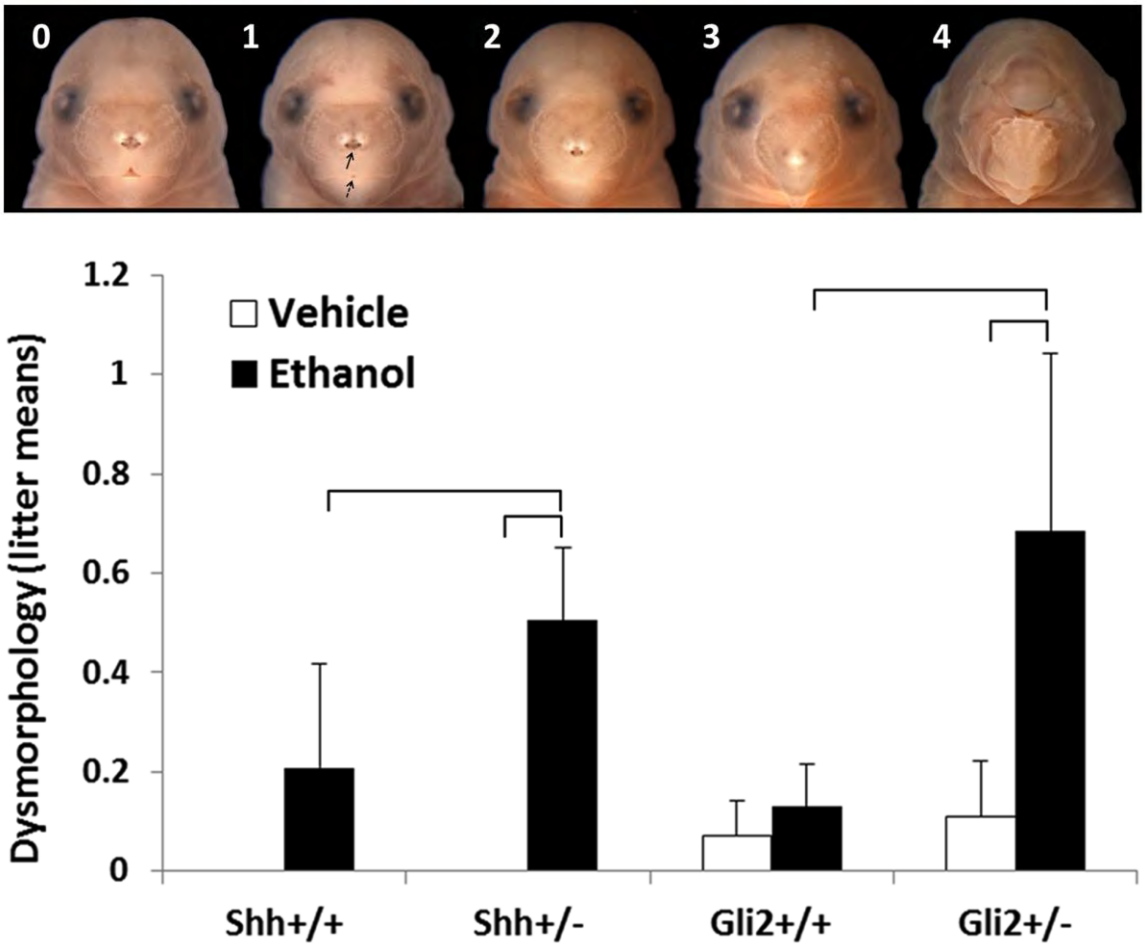


Figure 1. Illustrated above are a GD 17 fetus having normal facial morphology and 4 fetuses with varying degrees of medial facial deficiency. Numbers assigned to each image (0–4) are scores representing differing degrees of severity of facial dysmorphology. As compared to normal fetuses, those receiving a score of 1 had a notably diminished area of pigmentation between the nostrils (solid arrow) accompanied by reduction in the depth of the normally present median central notch of the upper lip (dashed arrow). A score of 2 was assigned to fetuses that had lost the median lip notch, but still had some remaining pigment at the tip of the nose. Individuals presenting with a single central nostril were assigned a score of 3 and those given a score of 4. For the graphed data shown, the average dysmorphology score from each genotypic group was determined for each litter in the study population. Values represent the mean plus the standard error of litter averages for each genotype and treatment. Brackets indicate p values of ≤ 0.05 as determined by a one-tailed student's t-test. From Kietzman et al, 2014

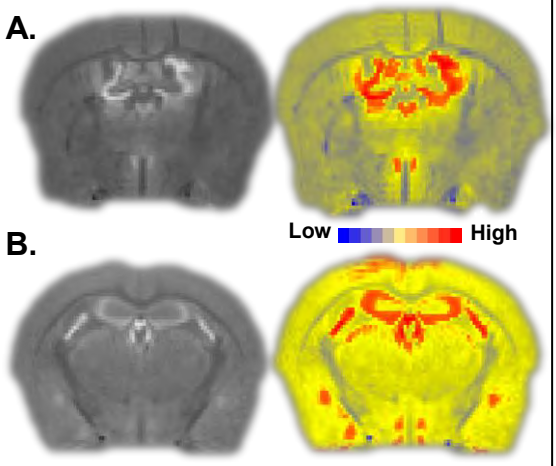


Figure 2. Representative images (T1-weighted images, left column; pseudo-colored images, right column) show high Mn^{2+} signal in the paraventricular nucleus **(A)** and the hippocampus and central nucleus of the amygdala **(B)** after 15 min of restraint stress.

During this reporting period, we have also initiated a study directed toward examining potential teratogenic interactions between alcohol and high potency cannabinoids. The rationale for this work lies in the premise that alcohol and cannabinoids are commonly concurrently used and that this co-drug exposure (environment/environment interaction) will yield more severe teratogenic outcomes than each agent alone. This work also rests on the fact that new "designer" cannabinoids are extremely potent and little teratogenicity data exists for them. Pilot data collected to date employing administration of the synthetic cannabinoid, CP-55,940, on GD 7 or 8 in mice illustrate induction of craniofacial malformations including anophthalmia, microphthalmia, iridial coloboma, exencephaly, midfacial deficiencies consistent with holoprosencephaly, and median facial clefting. These defects are consistent with those that result from alcohol exposure at these same time periods.

Finally, progress toward identification of fiber tract changes following GD 7 alcohol exposure includes completion of the MR scanning for 48 adult mice, with data in the pipeline for analyses. Importantly, the structural analyses are being coupled with functional endpoints. To further our understanding of structure/function relationships we are hoping to apply Magnesium Enhanced MRI (MEMRI) techniques to our model system. Pilot studies to date on control adult mice following restrain stress are promising, with stress resulting in signal in expected brain regions (Figure 2).

Discussion and Significance: A highlight of our work during this time period is completion and publication of our Shh and Gli2 mutant mouse study. This study has provided a springboard for additional gene/environment studies and for more in depth examination of the interaction of alcohol with genes in, or affecting the Shh pathway. We are particularly excited to initiate studies employing the Smoothened agonist, SAG, as this agent is expected to ameliorate alcohol's teratogenicity. As further discussed in the attached supplement progress report, we are also excited to extend our current work by beginning to look at potential teratogenic interactions between alcohol and high potency cannabinoids. We feel that this work is timely, important for the FASD field, and in keeping with our primary objective of making clinically significant discoveries regarding prenatal alcohol (ethanol) exposure-induced pathology involving the brain and face. In addition to these gene/environment and environment/environment studies, establishment of the MEMRI technique for the study of alcohol-exposed mouse brains has been a major step toward analyses of functional endpoints.

Interrelation with the Aims of the Consortium and Other Projects: Our correlative brain/face studies complement those of Dr. Faroud's group and we will continue to work closely with Dr. Peter Hammond and Mike Suttie on the analyses of facial dysmorphology induced by stage-dependent alcohol insult. Our brain imaging studies complement those of Dr. Sowell and Dr. Wozniak. And, our studies assessing genetically modified mice and their susceptibility to alcohol teratogenesis complement and extend the work of Dr. Johann Eberhart.

Plans for the next year:

1. Work with Mike Suttie to complete data processing and analyses for GD 10 and 11.5 exposures and publish the results of this study
2. Follow up on gene/environment studies, examining Shh and Gli mutant mice following alcohol exposure on GD 8.5 and publish the results of this study.
3. Working with SAG, we hope to further illustrate the importance of Shh pathway perturbation and to potentially identify a new FASD ameliorative agent.
4. Continue pilot studies looking at the potential of "super" cannabinoids to interact with alcohol as a teratogen

5. Continue pilot MEMRI studies to supplement the MRI and DTI studies in providing structure/function correlates. Dr. Lindsay Wiezoreck anticipates conducting this work with the aid of F32 support, having submitted an application for the April, 2014 deadline.
6. Present RSA abstracts in June, 2014
7. Dr. Sulik will present an invited NOFAS webinar in August, 2014

Publications:

Full papers

1. Parnell SE, Holloway HT, O'Leary-Moore SK, Dehart DB, Paniaqua B, Oguz I, Budin F, Styner MA, Johnson GA, Sulik KK. (2013) Magnetic resonance microscopy-based analyses of the neuroanatomical effects of gestational day 9 ethanol exposure in mice. Neurotoxicol Teratol. Sep-Oct;39:77-83. PubMed PMID: 23911654; PMCID: PMC3795920.
2. Dou X, Wilkemeyer MF, Menkari CE, Parnell SE, Sulik KK, and Charness ME. (2013) Mitogen-activated protein kinase modulates ethanol inhibition of cell adhesion mediated by the L1 neural cell adhesion molecule. *Proceedings of the National Academy of Sciences of the United States of America*,110:5683-5688. PMCID:PMC3619378
3. Kietzman HW, Everson JL, Sulik KK, Lipinski RJ. (2014) The teratogenic effects of prenatal ethanol exposure are exacerbated by Sonic Hedgehog or Gli2 haploinsufficiency in the mouse. PLos ONE PMCID: PMC3929747
4. Langer L, Sulik K, and Pevny L. (2014) Cleft palate in a mouse model of SOX2 haploinsufficiency. *The Cleft Palate-Craniofacial Journal,* 51:110-104.
5. Parnell E, Holloway HT; Paniagua B, Oguz I, Styner MA, Johnson GA, Sulik KK. (2014, in press) Dysmorphogenic effects of first trimester equivalent ethanol exposure in mice: a magnetic resonance microscopy-based study. Alcohol Clin Exper Res

Publications Reported for this Reporting Period

NIH Public Access Compliance	Citation
Complete	Kietzman HW, Everson JL, Sulik KK, Lipinski RJ. The teratogenic effects of prenatal ethanol exposure are exacerbated by sonic hedgehog or gli2 haploinsufficiency in the mouse. PLoS One. 2014;9(2):e89448. PubMed PMID: 24586787; PubMed Central PMCID: PMC3929747.
In process at NIHMS	Langer L, Sulik K, Pevny L. Cleft Palate in a Mouse Model of SOX2 Haploinsufficiency. Cleft Palate Craniofac J. 2014 Jan;51(1):110-4. PubMed PMID: 23638914; NIHMSID: 574253.
Complete	Parnell SE, Holloway HT, O'Leary-Moore SK, Dehart DB, Paniaqua B, Oguz I, Budin F, Styner MA, Johnson GA, Sulik KK. Magnetic resonance microscopy-based analyses of the neuroanatomical effects of gestational day 9 ethanol exposure in mice. Neurotoxicol Teratol. 2013 Sep-Oct;39:77-83. PubMed PMID: 23911654; PubMed Central PMCID: PMC3795920.
Complete	Dou X, Wilkemeyer MF, Menkari CE, Parnell SE, Sulik KK, Charness ME. Mitogen-activated protein kinase modulates ethanol inhibition of cell adhesion mediated by the L1 neural cell adhesion molecule. Proc Natl Acad Sci U S A. 2013 Apr 2;110(14):5683-8. PubMed PMID: 23431142; PubMed Central PMCID: PMC3619378.

Abstracts and Presentations:

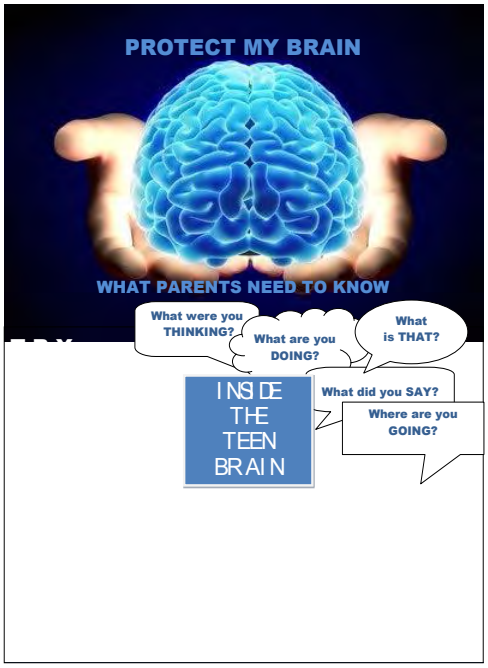
Abstracts

1. O'Leary-Moore SK, Budin F, Paniagua B, Oguz I, Johnson GA, Sulik KK. High-resolution neuroimaging reveals a range of corpus callosum insult induced by ethanol on gestational day 7 in mice. Alcohol Clin Exper Res, 37 suppl 2:167A, 2013
2. Kietzman HW, Sulik KK, Lipinski RJ. The effect of Shh and Gli2 heterozygosity in a mouse model of Fetal Alcohol Syndrome. Alcohol Clin Exper Res, 37 suppl 2:167A, 2013
3. Sulik KK. Prenatal alcohol exposure and abnormal brain development: Insights from animal studies. Proceedings of the FASD legal issues consensus development conference, Edmonton, Alberta Canada, September, 2013
4. Kietzman HW, Sulik KK, Lipinski RJ. The interaction of *Shh* and *Gli2* mutations with prenatal ethanol exposure in the genesis of fetal alcohol syndrome and holoprosencephaly. DW Smith Workshop on Malformations and Morphogenesis, Mt Tremblant, Canada, August, 2013
5. Wieczorek L, Budin F, Fish E, Sulik K. Application of manganese-enhanced magnetic resonance imaging to the study of the physiological effects of acute stress on prenatal alcohol-exposed mice. Alcohol Clin Exper Res submitted, 2014
6. Parnell SE, Baker LK, Sulik KK. Early gestational ethanol exposure induces altered seizure susceptibility in neonatal and adolescent mice. Alcohol Clin Exper Res submitted, 2014
7. Fish EW, Parnell SE, Gilbert MT, Sulik KK, Williams KP. Sonic Hedgehog Pathway Agonist-Induced Birth Defects: Implications for Fetal Alcohol Spectrum Disorders. Alcohol Clin Exper Res submitted, 2014
8. Lamson D, Bealer W, Tarpley M, Sulik KK, Williams KP. Modeling and analyzing ethanol-induced sonic hedgehog protein variants. Alcohol Clin Exper Res submitted, 2014
9. Gilbert MT, Parnell SE, Fish EW, Baker LK, Sulik KK. A mouse model shows synthetic cannabinoid teratogenicity and promise for prenatal drug co-exposure research. International Cannabinoid Research Society Meeting, submitted 2014

Presentations

K. Sulik participated in an FASD legal issues consensus development conference in Edmonton, Alberta Canada, in September, 2013. A video of her presentation is on the following site: http://www.ihe.ca/research/knowledge-transfer-initiatives/--consensus-development-conference-program/legal-issues-of-fasd/presentations-1/.

On October 12, 2013, K. Sulik gave a presentation on FASD for the Together for Resilient Youth Parent Conference In Durham NC (see event announcement below).



On March 6, 2014, K. Sulik presented a seminar at IUPUI, Stark Neuroscience Research Institute entitled “Imaging the Face and Brain in an FASD mouse model”

Book chapter:

1. Sulik KK, Fetal Alcohol Spectrum Disorder: Pathogenesis and Mechanisms, In: Pfefferbaum and Sullivan, Alcohol and the Nervous System 1E (Handbook of Clinical Neurology), Elsevier, 2014 (in press)

Supplement (AA021651-01S1)

Objectives and Goals: This supplement is directed toward promoting the health science training and career of Dr. Marcoita Gilbert. As funded, it supports both research and mentoring/career development activities. The primary objective of the basic research is to make clinically significant discoveries regarding prenatal alcohol (ethanol) exposure-induced pathology involving the brain and face. This work naturally builds on our CIFASD-supported basic research to date and continues to address the need for a more complete understanding of the spectrum and exposure stage-dependency of abnormalities caused by prenatal alcohol exposure. Our overall hypothesis is that alcohol induces structural abnormalities of the brain and face of mice that are consistent with and informative for those in human FASD.

Dr. Gilbert's accomplishments/activities during this funding period (6/01/2013-5/31/14) follow:

- Attended RSA meeting, Orlando, Florida, June 2013

- Took a leadership role in importing and establishing Lrp2 +/- mice for a new study conceptualized while meeting with Dr. Johann Eberhart at the January 2013 CIFASD meeting

- Drs. Allyn Howlett and Scott Parnell were invited to join Dr. Gilbert's professional development advisory committee

- Initiated a study to extend the laboratory's work with Shh and Gli2 mutant mice (see Kietzman et al 2014) to include examination of GD 8.5 alcohol insult. Worked with Dr. Eric Fish to re-establish the breeding colonies, genotyping and treatment protocols and to conduct Shh agonist experiments

- Initiated a study directed toward examining potential teratogenic interactions between high potency cannabinoids and alcohol. Worked with Dr. Fish to establish cannabinoid exposure paradigm.

- Participated in Sulik laboratory mouse neurobiology review course.

- Gave lab meeting presentations on cannabinoid teratogenicity and on teratogenic gene/environment interactions

- Attended Carolina Collaborative Cannabinoid Conference, Oct 2013

Discussion: Dr. Gilbert continues to make substantial progress in becoming well-versed in basic science and clinical issues related to FASD. This is providing a foundation for her current and future research. Importantly, she has successfully transitioned from her graduate research avian cannabinoid research model to employing a mouse model system. Regarding Dr. Gilbert's research to date, in addition to initiating studies outlined in her proposal, she has also taken the lead in planning and beginning to execute other studies of interest to her and that are in keeping with the CFASD goals. A study directed toward examining alcohol-exposed LRP2 knockout mice was conceptualized at the January 2013 CIFASD meeting following data presentation and discussion with Dr. Johann Eberhart. For this, Dr. Gilbert took the lead in acquiring LRP 2 knockout mice from Dr. Willnow in Germany. Unfortunately, the colony was not productive and we have found it necessary to drop this project. The Shh/Gli2 study that Dr. Gilbert is conducting promises to provide further support for the involvement of interference by

alcohol with the Shh signaling pathway. It also promises to aid our understanding of the developmental stage-dependent genesis of opposing facial/brain phenotypes (too narrow versus too wide). The cannabinoid/alcohol interaction study that Dr. Gilbert has initiated wonderfully combines her graduate cannabinoid research expertise with what may be a very important clinical FASD issue. The later being consideration of the combined adverse effect of high potency cannabinoids and alcohol on prenatal development. Pilot studies conducted in C57Bl/6J mice to date are very promising, with comparable facial and ocular abnormalities resulting from GD 7 or 8.5 cannabinoid exposure as result from alcohol by itself at these same time periods. Overall, Dr. Gilbert has demonstrated the initiative, enthusiasm and drive that exemplify a successful scientist.

Interrelation with the Aims of the Consortium and Other Projects: The gene/ environment studies being conducted complement those of the Eberhart and Faroud groups.

Plans for the next year:

- Attend RSA meeting in Seattle, WA, June 2014

- Attend International Cannabinoid Research Society Meeting, Baveno, Italy, June 2014

- Professional development advisory committee meeting

- Conduct and report results of Shh pathway mutant mouse studies

- Conduct cannabinoid/alcohol co-exposure studies

- Participate in grantsmanship course

Publications

1. Fish EW, Parnell SE, Gilbert MT, Sulik KK, Williams KP. Sonic Hedgehog Pathway Agonist-Induced Birth Defects: Implications for Fetal Alcohol Spectrum Disorders. Alcohol Clin Exper Res, 2014, submitted abstract
2. Gilbert MT, Parnell SE, Fish EW, Baker LK, Sulik KK. A mouse model shows synthetic cannabinoid teratogenicity and promise for prenatal drug co-exposure research. International Cannabinoid Research Society Meeting, 2014, submitted abstract