Spring 2015 Internal CIFASD Progress Reports
Rockville, MD March 19 & 20, 2015

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Principal Investigator(s): Edward P. Riley
Institution(s): San Diego State University
CIFASD Project Title: Administrative Core of the CIFASD
Grant Number: 5 U24 AA014811-11

1. What are the **major goals** of the project?

Specific Aims. The overall goals of the Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD) are to further refine definitive diagnoses of fetal alcohol spectrum disorders (FASD) at different stages of the lifespan based on biological, physical, neurological, and/or behavioral assessment. The CIFASD proposes a comprehensive approach to tackle many of the critical questions in FASD research, as outlined in the Request for Applications (RFA): improved diagnosis of FASD; enhanced understanding of dysmorphology (using 2-D and 3-D image analyses), neurobehavioral phenotype, and neuropathology associated with FASD; and enhanced early case identification so that interventions can be initiated as early as possible. We are integrating information from three modalities (brain, face, and behavior) at a variety of ages, and including basic animal studies, to guide early recognition of FASD with greater sensitivity.

The primary role of the Administrative Core (AdminC) is to serve a central coordinating role for CIFASD, ensuring that all projects proceed efficiently and results are shared across all projects. To that end, the Specific Aims of the AdminC remain primarily unchanged.

Aim 1. Provide scientific and administrative direction, leadership and oversight to the CIFASD. The PI coordinates interactions among the various projects and ensures that CIFASD investigators adhere to the goals and mission of the consortium. The AdminC also provides assistance and necessary administrative support to the Science Advisory Board (SAB) and CIFASD investigators, acting as the main liaison among the SAB, investigators, and NIAAA.

Aim 2. Facilitate communication among the various projects using the CIFASD website, scheduled monthly conference calls, biannual meetings, and the preparation and distribution of annual progress reports. Additionally, an Educational Component has been added to the CIFASD, to make it similar to a P60 Comprehensive Center for Excellence. This will allow for the wider dissemination of scientific knowledge directed to the public, patient populations, policy makers, and professionals.

Aim 3. Provide assistance where necessary with data collection and ensure that data from the projects are uploaded into the Central Repository in a timely fashion, so that consortium data may be accessed by all CIFASD projects and, eventually, outside projects. The PI works with the Informatics Core to develop online interactive capacity among CIFASD investigators, and assists outside investigators with CIFASD materials.

Aim 4. Assist the SAB and CIFASD investigators in their annual evaluations of progress. In conjunction with the SAB, the AdminC establishes annual priorities and manages issues related to the allocation of resources.

Aim 5. Oversee the Developmental Component of the CIFASD, from project solicitation to selection to completion and to be responsible for the Educational Component.

2. What was **accomplished** under these goals?

Major Activities. The Collaborative Initiative on FASD is a multisite, multidisciplinary consortium to address the issue of fetal alcohol spectrum disorders utilizing both basic and clinical research paradigms. The Administrative Core has the responsibility of providing the necessary administrative and scientific leadership to achieve the aims outlined in the original application.

Specific Objectives. Provide scientific and administrative leadership and oversight, ensuring that CIFASD investigators adhere to its goals and mission, as well as assisting and coordinating interactions between the various projects. Ensure that the Science Advisory Board and NIAAA advisors are consulted on matters related to both the science and administration of CIFASD and to incorporate their feedback into the overall mission of CIFASD. Facilitate communication between the various projects by maintaining the CIFASD website, moderating monthly conference calls, convening the biannual meetings of the CIFASD, and preparing the annual progress reports. Ensure the collaboration and interaction of the various projects in meeting their specific scientific goals and the integrated aims. Provide assistance with data collection and sharing by working with the Informatics Core. Administer the Developmental Projects of the CIFASD. Facilitate the communication of CIFASD findings to the scientific community, policy makers, and the general public. Oversee the Educational Component of CIFASD.

Significant Results. The Administrative Core continues to coordinate the monthly CIFASD conference calls via AccuConference and WebEx. WebEx was incorporated last year into the conference calls, to provide a platform for the distribution of findings from the various CIFASD projects. Each month a CIFASD investigator is invited to present their research to the other investigators. This has proved beneficial in promoting the integration of the various projects. The Admin Core also continues to assist the conferencing of smaller workgroups within CIFASD (e.g. publications and data access working groups) to provide new guidelines to the CIFASD.

Following the April 2014 face-to-face meeting, the Data Access Committee and Publications Committees were charged with new goals to meet the consortium's needs and requirements. The committees developed the required forms needed to accomplish their goals. During this reporting period, online uptake forms were developed to allow outside investigators to request access to CIFASD data and a concept proposal system was developed to help facilitate interaction amongst CIFASD investigators on manuscripts in preparation. A data sharing policy is now available on our website (http://cifasd.org/datasharing/DataSharingPolicy.pdf) as is a revised publication policy. We have already begun to receive inquiries about data sharing. Dr. Thomas is the liaison between the committees and our website's administrator.

Our two day annual meeting, held in Rockville, MD in April 2014, proved to be a success and was attended by 31 individuals affiliated or invited by CIFASD. Dr. Philip Shaw from NHGRI, was one of our invited speakers who discussed *Insights into ADHD from Structural Neuroimaging,* a topic with overlap in findings with those coming out of CIFASD projects. Our annual progress report was distributed to all investigators, NIAAA, and the SAB prior to the meeting. The Admin Core is currently in the final stages of preparation for our mid-March 2015 meeting. The agenda will include an overall discussion of how CIFASD should move into its next phase as this competitive renewal ends 2017.

The Informatics Core continues to provide the Admin Core monthly summaries of data entered into the Central Repository. We now have data on over 2,500 individuals in the repository, close to 1,000 3D facial images, over 200 brain scans, and nearly 500 neurobehavioral assessments on the current Phase III battery. We are pleased to report that almost all of the CIFASD sites are at or above their targeted goals. The Admin Core also maintains monthly updates on progress on Google Drive spreadsheets and graphs, which are available to all CIFASD investigators and advisors.

With the assistance of the CIFASD Science Advisory Board, an important undertaking of the Admin Core in the winter of 2014/15 has been the organization and management of the selection process for new developmental projects scheduled to begin on June 1, 2015. A call for letters of intent was posted on our website (http://cifasd.org/) and distributed in early November via the following listservs: RSA, FASD Study Group, ISBRA, ISDP, NADIA, DEARC and the

Teratology Society. The one-page letters of intent (LOI) detailed brief descriptions of the proposed research, the anticipated length and direct costs of the project, details on how the project would interrelate with the current goals of CIFASD, and a listing of any required CIFASD resources. Thirty-seven LOIs were received on December 1, 2014 and subsequently reviewed and evaluated by members of the SAB on their fit with CIFASD's mission and current projects, as well as their scientific merit. By early January, all LOI PIs were notified as to whether or not their project was still under consideration and the pool of possible projects was triaged down to sixteen; these projects were invited to submit a more developed research strategy (maximum of 5 pages) as well as detailed budgets by February 8, 2015. These more developed proposals have been sent out to external reviewers (coordinated by Drs. Riley and Thomas) to be ranked on their scientific merit, significance, fit with the consortium, and principal investigator. Each proposal is also being reviewed by a current member of CIFASD to comment on its feasibility and fit. The goal is to have the field of sixteen narrowed down to approximately five possible projects by early March and to have the final selections determined by the end of March. This will give the selected projects two months to secure their IRB and/or IACUC approvals. It is possible some of the proposed developmental project PIs will be invited to the March face-to-face meeting to present their research ideas to the group. If possible, the scope of work, timeline, and budget pages of the selected project will be included in the NIH RPPR due April 1, 2015.

Upon submission of the Federal Financial Report by SDSURF, a carryover request was submitted and received in October 2015. Additional time was requested for developmental projects to complete their aims and their subcontracts were set-up by the Admin Core. Additionally, the Admin Core created and distributed templates for the new NIH progress reports due April 2015. The Advisory Board will review these internal progress reports, data collection progress charts/graphs, and the project's PPT presentations at the upcoming meeting to complete their formal evaluations of each project. The format of our internal progress reports has been modified to follow the NIH RPPR format, as our U24 and U01 projects will be using the NIH RPPR format for their formal progress reports due April 1, 2015. Templates were created by Administrative Core personnel and distributed to all project PIs. The Advisory Board will review these internal progress reports, data collection progress charts/graphs, and the project's PPT presentations at the upcoming meeting to complete their formal evaluations of each project.

Key Outcomes. Numerous specific objectives have been met by the Admin Core and the other U24 Cores, and the U01 Projects have been doing an outstanding job in meeting their target goals. However, I believe the key outcome of the Admin Core during this reporting period has been the improved integration of the projects. Numerous publications from CIFASD now deal with the interrelatedness of data across projects. Our efforts towards interrelating changes in face, brain and behavior in both clinical and basic science studies have been fruitful and are leading to new insights regarding the mechanisms of damage, enhanced diagnostic capabilities, understanding the behavioral profiles of FASD, and learning more about the underlying brain changes in FASD.

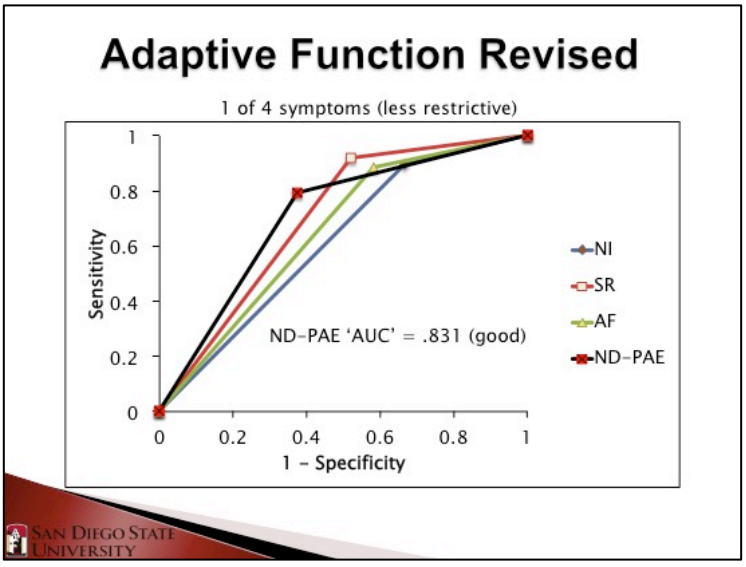
3. For this reporting period (April 1, 2014 - March 1, 2015), is there one or more **Revision/Supplement** associated with this award for which reporting is required?

The Admin Core also received a supplement, 3U24AA014811-11S1, from NIAAA during the current reporting year entitled, "ND-PAE Administrative Supplement" to support two sets of secondary analyses on existing data from the CIFASD consortium. The first analysis is attempting to validate the newly developed Neurobehavioral Disorder Associated with Prenatal Alcohol Exposure (ND-PAE) diagnostic category in DSM-5. The second analysis would be to apply separately, the Hoyme 2005 criteria, the Canadian 2005 criteria, and the 4-digit Code

criteria to existing data for comparative estimation of FASD diagnoses to clarify differences in FASD estimates using these three diagnostic schema.

Since the receipt of supplemental funds to examine the ND-PAE criteria using the CIFASD neurobehavioral data from U01 AA01834 (S. Mattson, PI), we have made considerable progress toward the aims of the supplement. We hired a statistician, Dr. Melody Sadler (SDSU), who has worked closely with Dr. Mattson on these analyses. While still in progress, thus far we have:

- Identified the specific variables from CIFASD II data that match the criteria. This was largely done for the proposal, but was modified slightly and finalized. We also worked closely with Dr. Julie Kable who is working on a parallel analysis on a different dataset to make sure we are working in a consistent fashion.
- Downloaded and cleaned the data to prepare it for analysis.
- Tested the reliability of the diagnostic domains within the alcohol-exposed (AE) group, using both 1 standard deviation (SD) and 1.5 SD cutoffs, as proposed. Adequate reliability (a > .78 at both SD cutoffs) was found for all domains. Adequate reliability of symptoms within domains was also adequate for the neurocognitive impairment and self-regulation domains, but somewhat lower for the adaptive functioning (AF) domain but would increase if motor impairment were not included.
- Tested the relation between domain and diagnosis within the AE group. As originally proposed, the AF domain was highly correlated with diagnosis (r > .88 at both SD cutoffs). If modified to require only 1 AF criteria, instead of 2, the correlations drop and are more equivalent across domains.
- Tested group discrimination using the ND-PAE criteria. We identified two group comparisons – AE vs. CON and AE vs. expCON. The CON group was our original CON group from CIFASD II, it is exclusive of children with IQ<88 or with ADHD. The expCON group includes these children and provides a more heterogeneous comparison group. We tested 5 statistics: sensitivity, specificity, positive predictive values (PPV), negative predictive values (NPV), and receiver operator curves (ROC, see figure). All of these variables are significant for the 1.0 and 1.5 SD cutoffs and results are improved with the modification of the AF criteria. We are currently examining the data more closely to determine how to improve our results even further. For example, we plan to test whether relaxing the IQ criterion (currently requires a 2SD cutoff) will improve our results. We also plan to test our results with a 2SD cutoff across the board in order to address differences with the Canadian guidelines.



4. What **opportunities for training and professional development** has the project provided?

Nothing to Report.

5. How have **results** been **disseminated** to communities of interest?

Much has also been accomplished toward our outreach and educational goals. Dr. Charness, the Scientific Director of CIFASD, organized a symposium for the joint RSA/ISBRA meeting entitled, "CIFASD Studies of Genetic Susceptibility to Fetal Alcohol Spectrum Disorders," which included presentations from CIFASD investigators Eberhart, Dou, Hammond, and Wetherill. This symposium was well-attended and provided exposure on the consortium's accomplishments to an international audience. CIFASD also recently submitted a symposium entitled, "The Face, Brain, and Behavior in FASD: Connecting the Dots in CIFASD," which was accepted for presentation at the 2015 RSA meeting. This symposium will include Drs. Charness, Mattson, Sowell, Hammond and Foroud. The CIFASD was invited back to present a half-day plenary for the 6th International Conference on FASD in Vancouver, Canada to be held in early March 2015. The plenary will include presentations by Drs. Riley, Eberhart, Charness, Parnell, Hammond, Foroud, Sulik, Uban, and Warren. Other CIFASD investigators are presenting in separate symposia at this conference as well (Chambers, Mattson).

Numerous CIFASD investigators have also given webinars during the past year on their research and these are available at http://cifasd.org/education/ and http://www.nofas.org/nofas-webinar-series/.

Dr. Riley continues to present at meetings on CIFASD and its accomplishments. As an example, he presented CIFASD research in conjunction with an NIAAA trip to Estonia, Latvia and Lithuania. He also presented at the EUFASD and APSAAR meetings during the last year.

For the progress or the Educational Component of CIFASD (NOFAS), please see their individual progress report.

6. What do you **plan to do for the next reporting period** (March 1, 2015 - April 1, 2016) to accomplish the goals?

At a minimum, two new developmental projects will begin June 1, 2015. It is possible carryover funds will be used to fund additional developmental projects and/or administrative supplements. The Admin Core will prepare the carryover request and establish the subaward contracts. As June 1, 2015 will mark the start of the forth year of this renewal period, a main task for the PI of the Admin Core during the next reporting period will be to determine the charge and content of the next iteration of CIFASD.

7. Describe your project's **interrelation** with aims of the CIFASD consortium its other projects.

By its very nature, the Admin Core interrelates with each of the CIFASD projects.

8. **Publications** **[Accepted & In Press]**

CIFASD researchers continue to publish their results in scientific journals. During the current reporting period, CIFASD investigators published 16 papers and presented numerous abstracts. Importantly, in 2013 and 2014, six publications include CIFASD as a group authorship, demonstrating the integrative nature of our consortium. The PubMed-generated publication list on our website now lists 155 publications from CIFASD grants.

Publications listing the AdminC as a source of support this reporting period:

NIH Public Access Compliance	Citation
In process at NIHMS	Infante MA, Moore EM, Nguyen TT, Fourligas N, Mattson SN, et al. Objective assessment of ADHD core symptoms in children with heavy prenatal alcohol exposure. Physiol Behav. 2014 Oct 23;PubMed PMID: 25447751; NIHMSID: NIHMS642957. [Epub ahead of print]
Complete	Balaraman S, Lunde ER, Sawant O, Cudd TA, Washburn SE, et al. Maternal and neonatal plasma microRNA biomarkers for fetal alcohol exposure in an ovine model. Alcohol Clin Exp Res. 2014 May;38(5):1390-400. PubMed PMID: 24588274; NIHMSID: NIHMS567198; PubMed Central PMCID: PMC3999266.
Complete	Ware AL, Glass L, Crocker N, Deweese BN, Coles CD, et al. Effects of prenatal alcohol exposure and attention-deficit/hyperactivity disorder on adaptive functioning. Alcohol Clin Exp Res. 2014 May;38(5):1439-47. PubMed PMID: 24655090; NIHMSID: NIHMS567036; PubMed Central PMCID: PMC3999227.
Complete	Nguyen TT, Glass L, Coles CD, Kable JA, May PA, et al. The clinical utility and specificity of parent report of executive function among children with prenatal alcohol exposure. J Int Neuropsychol Soc. 2014 Aug;20(7):704-16. PubMed PMID: 25033032; NIHMSID: NIHMS620944; PubMed Central PMCID: PMC4228981.
Complete	Lovely CB, Eberhart JK. Commentary: catching a conserved mechanism of ethanol teratogenicity. Alcohol Clin Exp Res. 2014 Aug;38(8):2160-3. PubMed PMID: 25156611; NIHMSID: NIHMS596773; PubMed Central PMCID: PMC4147959.
Complete	Moore EM, Migliorini R, Infante MA, Riley EP. Fetal Alcohol Spectrum Disorders: Recent Neuroimaging Findings. Curr Dev Disord Rep. 2014 Sep;1(3):161-172. PubMed PMID: 25346882; NIHMSID: NIHMS603803; PubMed Central PMCID: PMC4207054.
Complete	Dou X, Charness ME. Effect of lipid raft disruption on ethanol inhibition of l1 adhesion. Alcohol Clin Exp Res. 2014 Nov;38(11):2707-11. PubMed PMID: 25421507; NIHMSID: NIHMS626802; PubMed Central PMCID: PMC4278581.

9. **Publications [In Preparation & Submitted]**

Mentioned in separate Developmental Project progress reports.

10. **Poster Abstracts and Presentations**

The PI gave talks at the following conferences and meetings this reporting period:

- April 2014 6th National Biennial Conference on Adolescents and Adults with FASD Vancouver, Canada
- April 2014 APSAAR Shanghai, China
- May 2014 Alcohol and Stress Conference Volterra, Italy
- September 2014 Baltic States (Lithuania, Latvia and Estonia)
- October 2014 EUFASD Rome, Italy
- November 2014 AG7 FASD Conference Sudbury, Ontario, Canada

Principal Investigator(s): Tom Donaldson and Kathy Mitchell
Institution(s): National Organization on Fetal Alcohol Syndrome (NOFAS)
CIFASD Project Title: Educational Component of the Administrative Core of the CIFASD
Grant Number: U24 AA014811 (Administrative Core of the CIFASD)

1. What are the **major goals** of the project?
The major goals of the NOFAS educational component are to 1) increase the awareness of the findings of CIFASD and of the overall significance of CIFASD and its research among diverse audiences both nationally and internationally, and 2) increase the use of FASD findings as the evidence-base for enriching public health prevention and clinical intervention strategies.

2. What was **accomplished** under these goals?
NOFAS is meeting the project goals though a Webinar Series featuring CIFASD scientists, presentations to professionals and lay audiences, briefings for NOFAS partners and—upon request—policymakers, and media strategies. Each activity is designed to meet one or more of the specific objectives described in this section.

Major Activities during the reporting period include:
NOFAS Webinar Series – The monthly webinar series hosted by NOFAS features CIFASD scientists and their research. The webinars bring CIFASD findings to a range of professionals within and outside of the FASD field and to families and caregivers living with the disorders. The following presentations are among those that were held or are scheduled during the reporting period:

- Michael Charness, MD - *An Update on Fetal Alcohol Spectrum Disorders and the Risk of Light Drinking During Pregnancy*
- Kathy Sulik, PhD - *Animal Model-based FASD Research: Insights Regarding Alcohol-induced Abnormal Development*
- Christina Chambers, PhD - *How does mother's nutrition affect pregnancy outcome in women who drink alcohol?*
- Kenneth Lyons Jones, MD - *Alcohol and Pregnancy: What Have We Learned in 40 Years?*
- Jeff Wozniak, PhD – *Title to be determined* – Scheduled for March 2015

Presentations – NOFAS presentations, meetings, and briefings emphasize CIFASD content and provide an overview of CIFASD goals.

April 2014

- Presented a workshop: *The Wisdom of Experience: The Dual Lives of Three Mothers Making a Difference* at the 6th National Biennial Conference on Adolescents and Adults with FASD: Changing the Conversation, Vancouver, BC.
- Presented a one-day workshop: *Fetal Alcohol Spectrum Disorders (FASD): A Frequently Overlooked Diagnosis of Children with Learning and Behavioral Disabilities* for the staff from Early Education Services, Brattleboro, VT.
- Presented a two-hour Grand Rounds: *Creating a Circle of Hope: The Role of the Physician in the Identification and Prevention of Fetal Alcohol Spectrum Disorders (FASD),* Brattleboro, VT.
- Lectured for the Physician Assistant Program, School of Medicine and Health Sciences, The George Washington University, Washington, D.C.
- Lectured at the Georgetown University Nursing Program, Washington, D.C.

May 2014

- Presented a one-day workshop: *Fetal Alcohol Spectrum Disorders (FASD): A Frequently Overlooked Diagnosis of Children with Learning and Behavioral Disabilities,* for teachers at Garrett College, Garrett County, MD.
- Facilitated a one-day workshop: *Fetal Alcohol Spectrum Disorders (FASD): Creating Pathways to Prevention, Intervention & Support;* sponsored by NOFAS-Colorado for educators and other professionals, Denver, CO.
- Co-facilitated a webinar *FASD: Creating a Circle of Hope* sponsored by the Indian Health Service (IHS) and the University of New Mexico Office of Continuing Medical Education and the UNM Center for Rural & Community Behavioral Health.

June 2013

- Provided a keynote: *Creating a Circle of Hope* for the Florida Fights FASD Conference sponsored by the Florida Center for Early Childhood, Sarasota, FL.
- As requested, briefed staff members for Representatives Tom Cole, Mike Honda, and Don Young on CIFASD and FASD.

August 2013

- Facilitated a Webinar: *Introduction to Fetal Alcohol Spectrum Disorders (FASD) and Child Welfare Practice* for Maryland child welfare professionals, sponsored by the Child Welfare Academy, University of Maryland, School of Social Work, Baltimore, MD.
- As requested, briefed aides to Senators Lisa Murkowski, Tom Harkin and Tammy Baldwin and Representatives Rosa DeLauro and Lucille Roybal-Allard on CIFASD and FASD.

September 2013

- Provided the keynote address: *Creating a Circle of Hope*, and a workshop *FASDs: It Takes a Community* for the Oklahoma Child Abuse and Neglect Conference in collaboration with the Oklahoma Drug Endangered Children Alliance, Norman, OK
- Provided the keynote address: *Creating a Circle of Hope* for the 2nd Annual District of Columbia FASD Conference; sponsored by Howard University College of Medicine and District of Columbia Department of Health and Human Services and NOFAS.
- Facilitated a workshop: *Fetal Alcohol Spectrum Disorders (FASD): An Invisible Disability* for Maryland lawyers at the 2014 Eleventh Annual Child Abuse and Neglect Attorney Conference sponsored by the Foster Care Court Improvement Project, Annapolis, MD.

November 2013

- Provided a one-day workshop *FASD; Modifying Systems of Care for Improved Outcomes* for staff from the Center for Social Change, Elkridge, MD.
- Presented the keynote address to the Texas FASD Collaborative and state lawmakers at the Texas Capitol Building, Austin, TX.

January 2014

- Presented a two-hour workshop: *Supporting Women to Prevent FASD in their Offspring*; for staff from the Partners with Mom Program, Johns Hopkins HealthCare, Baltimore, MD.

February 2014

- Presented a webinar: *Resiliency in Families Living with FASD* for the Frontier Regional FASD Training Center, Center for the Application of Substance Abuse Technologies (CASAT), University of Nevada, Reno, Reno, NV

NIAAA Twitter Chat – NOFAS conducted a Twitter Chat in collaboration with NIAAA on International FASD Awareness Day.

NOFAS Excellence Award – NOFAS presented the Excellence Award to Michael Charness, MD at the June NOFAS Awards Reception.

The **specific** project **objectives** are:

- Promote CIFASD scientists and findings through a minimum of six webinars
- Educate discipline-specific practitioners, school administrators and teachers, and public health professionals, about CIFASD findings through a minimum of 12 in-person presentations and trainings reaching over 1,200 professionals
- Educate students, local and state officials, NOFAS partners, and other FASD stakeholders and lay audiences about CIFASD findings through a minimum of six in-person presentations or trainings reaching over 300 individuals
- Promote CIFASD, its findings, and cifasd.org through NOFAS communication channels, as warranted
- Promote CIFASD, its findings, and cifasd.org through NOFAS social media feeds, as warranted
- Maintain a CIFASD page on nofas.org
- Host one Twitter Chat with NIAAA
- Promote CIFASD, its findings, and cifasd.org in response to inaccurate coverage of FASD and to media inquires, as warranted

Significant Results. CIFASD findings are critical to the development of FASD clinical interventions, public health prevention initiatives, and applied research design. By increasing awareness of CIFASD and access to its findings, NOFAS ensures that the latest research serves as the evidence base that informs practical applications, advancing the field of FASD. As a result, individuals and families living with FASD, a primary NOFAS constituency, have the opportunity for the best possible outcomes.

Key Outcomes. All specific project objectives described previously in this section have been met or are on course to be met during the reporting period.

3. For this reporting period (April 1, 2014 - March 1, 2015), is there one or more **Revision/Supplement** associated with this award for which reporting is required? N/A

4. What **opportunities for training and professional development** has the project provided?
As described in the major activities section of this report, medical and allied health practitioners and students were involved in activities supported by the project as recipients of grand rounds or other trainings, and as a result attained greater proficiency in FASD and current research.

5. How have **results** been **disseminated** to communities of interest?
NOFAS regularly distributes CIFASD news and links to published findings through the NOFAS Weekly Roundup—an electronic FASD newsletter disseminated each Monday—and to followers of NOFAS social media feeds. NOFAS also disseminates CIFASD results through the 41-member NOFAS Affiliate Network. The Network consists of FASD organizations that diagnose, implement intervention strategies, host support groups, and/or conduct public health prevention initiatives. CIFASD scientists and/or their findings are featured in videos produced and materials designed by NOFAS that are disseminated at conferences, NOFAS presentations and meetings, and through the NOFAS Information and Referral Clearinghouse.

NOFAS disseminates CIFASD information and findings to partners such as the Centers for Disease Control and Prevention and its FASD grantees, including the newly funded CDC Practice and Implementation Centers, the SAMHSA FASD Center for Excellence, American College of Obstetricians and Gynecologists, American Academy of Pediatrics, and other partners.

6. What do you **plan to do for the next reporting period** (March 1, 2015 - April 1, 2016) to accomplish the goals?

NOFAS will continue to include information on CIFASD research and accomplishments in a range of public presentations and through the dissemination of both electronic (website, PPT slides, etc.) and print materials. NOFAS will also continue the NOFAS Webinar Series and conduct outreach to the media and policymakers. During the upcoming reporting period NOFAS will focus on incorporating CIFASD findings into new materials and resources for individuals living with FASD. NOFAS will also continue planning for an international FASD conference that would in part highlight CIFASD research and its scientists.

Scheduled webinars and presentations:

March 2014

- Scheduled for two oral presentations: *An International Campaign to Raise Awareness of the Risks of Drinking in Pregnancy* at the 6th National Biennial Conference on FASD Research: Results and Relevance 2015, Vancouver, B.C.
- Webinar – Jeff Wozniak, PhD – *Title to be determined* – Scheduled for March 2015
- Scheduled to lecture at the Georgetown University Nursing Program, Washington, D.C.

April 2015

- Scheduled to lecture at The George Washington University Physician Assistants Program, Washington, D.C.
- Scheduled to lecture at the Howard University School of Medicine, Washington, D.C.
- Scheduled to co-host a U.S. Congressional briefing with the Friends of NIAAA (NOFAS is an Executive Committee member of FoNIAAA, and recommended the topic of FASD and the presenters for a 2015 briefing).

7. Describe your project's **interrelation** with aims of the CIFASD consortium its other projects.

NOFAS brings CIFASD and its published research beyond academia and the scientific community by making the consortium's research relevant to targeted audiences of practitioners, educators, and other professionals and to lay audiences including the FASD community and the public at-large. NOFAS also provides CIFASD members with the perspective of the FASD family experience, the state of FASD policy objectives, gaps in services, public health challenges and other real world insight that can contribute to research aims.

8. **Publications [Accepted & In Press]** N/A

9. **Publications [In Preparation & Submitted]** N/A

10. **Poster Abstracts and Presentations**

NOFAS presented three poster sessions*:*

- Mitchell, K. *NOFAS: twenty five years of FASD prevention, media, and policy.* Presented at the 3rd EUFASD Conference, Rome, Italy, October 2014.
- Mitchell, K. *Creating a Circle of Hope: Supporting Birth Mothers to Prevent FASD.* Presented at the 3rd EUFASD Conference, Rome, Italy, October 2014.
- Mitchell, K. *The NOFAS K-12 Prevention Curriculum: An Evidence Based Model for Educating School Age Populations on FASD.* Presented at the 3rd EUFASD Conference, Rome, Italy, October 2014.

Principal Investigator(s): Johann K. Eberhart
Institution(s): University of Texas at Austin
CIFASD Developmental Project Title: Genetic Approaches to Understand Variability In FASD Facial and Neural Phenotypes
Grant Number: U24 AA014811 (Administrative Core of the CIFASD)

1. What are the **major goals** of the project?

Aim 1. Recover and characterize ethanol sensitive mutants identified in a forward genetic screen.

Aim 2. Screen community resources (zebrafish mutants house at ZIRC) to identify ethanol-sensitive loci.

2. What was **accomplished** under these goals?

Major activities. During this reporting period we have continued our efforts to clone and characterize ethanol-sensitive mutants obtained from our forward genetic screen and test candidate genes for sensitivity to ethanol.

Specific Objectives. A). Finish whole genome sequencing of ethanol-sensitive forward genetic mutants and B). Test candidate genes for ethanol sensitivity.

Significant Results. We have genome sequencing data for all 9 ethanol-sensitive mutants that were successfully recovered from our forward genetic screens. We are currently analyzing the sequence data for 6 mutants, have identified a candidate mutation in 1 line (*au33*) and have validated linkage to candidate mutations in 2 mutants. We anticipate having linked candidates for the 6 sequenced mutants within 2 months. We are currently designing genotyping protocols to determine if a SNP in *prpf31* is linked to *au33*. Of the 2 mutants with linked candidates, one is a mutation in a novel gene with similarity to *LDLR* and the second is in a poorly characterized gene, *armc10*. While virtually nothing is known about the function of *armc10*, a related gene, *ARMC4*, has been shown to cause ciliopathy when mutated in humans. Once the candidate genes are validated as being causative, we will submit a manuscript describing our forward genetic screen.

Based on discussion from the last CIFASD meeting we made screening of the cilia gene, *kif3a* a priority for this funding period. We obtained *kif3a* carriers and have tested them for interaction with ethanol. Preliminary data suggests an interaction. Untreated mutants have a curved body axis and slight defects to the lower jaw. In ethanol-treated clutches, approximately 50% of fish develop edema, a defect not observed in untreated clutches and craniofacial defects appear more prominent. We are currently establishing genotyping protocols to verify the interaction.

We have also continued our analysis of the genetic interaction between ethanol and members of the Wnt/PCP pathway that we identified in the previous grant cycle. Our analyses have focused on how ethanol-responsive miRNAs may mediate the interaction between ethanol and *vangl2*. From a published set of ethanol-responsive miRNAs, we found that several upregulated miRNAs were predicted to target members of the Wnt/PCP pathway. Of these, we have discovered that miR107 overexpression fully recapitulates the effect of ethanol on *vangl2* mutants. Furthermore, blocking miR107 function rescues development in ethanol-treated *vangl2* mutants. We are currently validating that miR107 is upregulated by our ethanol treatment paradigm.

Key Outcomes/Achievements. The most significant achievement of this funding period is achieving the ultimate goal of the development project: acquisition of R01-level funding. We submitted a new R01 proposal, which has received a 2% score. We anticipate funding later this year.

Our results have important implications for human health and FASD. Understanding the genetic underpinnings of susceptibility to FASD will inform diagnosis, prevention and therapy. Our zebrafish model has been extremely successful at predicting conserved gene-ethanol interactions in humans. Therefore, our current and future work to determine the mechanism of these gene-ethanol interactions will translate to human health. As we determine the most proximal alterations in cell signaling that lead to gene-ethanol interactions, we will gain insight into how to prevent such gene-ethanol interactions. Similarly, as we learn of the events downstream of these gene-ethanol interactions, we may well find ways to ameliorate the deleterious impacts of ethanol on development.

3. For this reporting period (April 1, 2014 - March 1, 2015), is there one or more **Revision/Supplement** associated with this award for which reporting is required?

N/A for Developmental Projects and/or Administrative Supplements

4. What **opportunities for training and professional development** has the project provided?

In addition to the conferences listed under abstracts, graduate students and postdocs receive professional development by attending and presenting in a weekly Wagonner Center group meeting of alcohol researchers and a bi-weekly Developmental Biology group meeting. Currently, trainees present work related to this project approximately once per year in each group meeting.

UT Austin is currently developing Individual Development Plans for personnel.

5. How have **results** been **disseminated** to communities of interest?

Nothing to report.

6. What do you **plan to do for the next reporting period** (March 1, 2015 - April 1, 2016) to accomplish the goals?

Not Applicable.

7. Describe your project's **interrelation** with aims of the CIFASD consortium its other projects.

We have interacted closely with Dr. Foroud and Dr. Hammond to apply our genetic findings to human. As we identify gene-ethanol interactions in zebrafish, we have searched for interactions within the human genetic dataset. These analyses identified ethanol-SNP interactions with members of the Pdgf, Bmp and Wnt/PCP pathway.

We have also interacted with Dr. Chambers in identifying potential candidates from human methylation data.

8. **Publications** **[Accepted & In Press]**

NIH Public Access Compliance	Citation
Complete	Lovely CB, Eberhart JK. Commentary: catching a conserved mechanism of ethanol teratogenicity. Alcohol Clin Exp Res. 2014 Aug;38(8):2160-3. PubMed PMID: 25156611; NIHMSID: NIHMS596773; PubMed Central PMCID: PMC4147959.

9. **Publications** **[In Preparation & Submitted]**

McCarthy, N., Bertrand, J. and Eberhart J.K. The role of Fibroblast growth factor signaling in the morphogenesis of the zebrafish postchordal neurocranium, eLife, Submitted.

10. **Poster Abstracts and Presentations**

Lovely, C.B., McCarthy, N., Sidik, A., Swartz, M.E. and Eberhart, J.K. Using Zebrafish to Identify and Characterize Gene-Ethanol Interactions. Experimental Biology, San Diego, April 2014.

McCarthy, N., Swartz, M.E., Lovely, C.B. Wells, M.B., Griffin, M., McGurk, P., Rozacky, J and Eberhart, J.K. Using Genetic Screens in Zebrafish to Identify Ethanol-Sensitive Loci. 37th RSA/17th Congress of ISBRA, Bellevue, June 2014.

Sidik, A. and Eberhart, J.K. Attenuation of the Planar Cell Polarity Pathway May Cause Susceptibility to FASD. 37th RSA/17th Congress of ISBRA, Bellevue, June 2014.

Lovely, C.B., Henegar, T., McCarthy, N., Swartz, M.E., and Eberhart, J.K. Zebrafish Genetic Screens Identify Ethanol Susceptibility Loci. 37th RSA/17th Congress of ISBRA, Bellevue, June 2014.

McCarthy, N. and Eberhart, J.K. Fgf8a Synergistically Interacts with Ethanol to Perturb Skull Development. 37th RSA/17th Congress of ISBRA, Bellevue, June 2014.

McCarthy, N. and Eberhart J.K. Disease Variability Caused by Gene-Environment Synergy. 10th Structural Birth Defects Meeting, Washington DC, December 2014.

Lovely, C.B., Henegar, T., Norrie, J.L., Swartz, M.E., and Eberhart, J.K. Zebrafish Genetic Screens Identify an Ethanol Susceptibility Locus. 25th Texas Research Society on Alcoholism Scientific Conference, Bryan, Feb 2015.

Sidik, A. and Eberhart, J.K. Attenuation of the Planar Cell Polarity Pathway May Cause Susceptibility to FASD. 25th Texas Research Society on Alcoholism Scientific Conference, Bryan, Feb 2015.

Principal Investigator(s): Dipak K. Sarkar
Institution(s): Rutgers, the State University of New Jersey
CIFASD Developmental Project Title: Circadian Genes, Stress Axis and Fetal Alcohol Spectrum Disorder
Grant Number: U24 AA014811 (Administrative Core of the CIFASD)

1. What are the **major goals** of the project?

The major goal of this developmental project is to measure changes in the DNA methylation of the stress regulatory circadian gene (period, PER) and proopiomelanocortin (POMC) gene as well as plasma cortisol levels using the biological fluid samples of FASD (FAS and fetal alcohol exposed) and control patient children from the CIFASD. Once available, we will compare gene and hormone data of subjects classified as FAS, alcohol exposed and control patient children. Individuals with FAS and alcohol exposed will be longitudinally assessed. The questions to be addressed here: Do methylation levels of PER2 and POMC genes correlate with the stress hormone abnormality (cortisol)? Furthermore, if these gene hypermethylation changes correlate with stress hormone abnormalities, are they manifested in patients with FASD? These studies will be crucial in determining the stability of the identified epigenetic signature of FASD patients and whether epigenetic signatures identified allow for earlier identification of alcohol-related birth outcomes.

2. What was **accomplished** under these goals?

Previous studies that led to development of the hypothesis being tested in the developmental project: Epigenetic modifications of a gene have been shown to play a role in maintaining a long-lasting change in gene expression. Our data in laboratory animals indicated that early-life alcohol feeding in rodents induces *Per2* gene hypermethylation and suppresses *Per2* mRNA levels in the hypothalamus, lowers *Pomc* mRNA expression in the hypothalamus, elevates corticosterone level in plasma, and increases alcohol drinking behaviors during adulthood. These led us to hypothesize that alcohol's modulating effect on DNA methylation makes a long-lasting epigenetic mark on *Per2* and *Pomc* genes that serves to activate the neuroendocrine stress axis via suppression of the *Pomc* gene; this facilitates maladaptation of the stress system. To test this in humans, in collaboration with Dr. Rajita Sinha (Yale University) we studied whether the interaction between alcohol, PER2 and POMC genes and the HPA axis exist in human. Using 30 light social drinking controls matched to treatment engaged alcohol dependent individuals, we found greater methylation of the PER2 DNA and POMC DNA in blood sample in alcoholics. Also, increased methylation levels were significantly associated with higher stress-induced cortisol. These data support the evidence obtained from animal studies that epigenetic modification of PER2 DNA and POMC gene may mediate alcohol modulation of HPA axis functions. Hence, we hypothesize that maternal alcohol consumption during pregnancy will make epigenetic marks on PER2 and POMC genes that will be manifested both in mother and offspring by demonstrating a significant increase in PER2 and POMC gene methylation and stress hormones (cortisol) levels in biological fluid. This hypothesis is tested in this CIFASD Developmental Project.

Status of accomplishment of the goals of the developmental project: We received salivary DNA samples of FAS, alcohol exposed and control male and female patients (7-16 years old) obtained from two separate trials in Dr. Tatiana Foroud's laboratory at the University of Indiana. Additionally, we received DNA samples from the same patients that were used in the first trial for conducting longitudinal studies. We measured DNA methylation levels of PER2, PER1 and POMC genes in saliva using a methylation specific PCR (MS-PCR) assay. We found that 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 two separate trials. Determination

of the stability of gene methylation in a longitudinal study also showed that increased methylation levels of PER2 and POMC persist in the same patients in a follow up study. The alcohol effect on PER gene methylation appears to have some specificity, since PER2 and not PER1 gene methylation differ between FAS, alcohol exposed and control subjects. To validate the MS-PCR data on DNA methylation, we also used a pyrosequencing assay, which can accurately provide real-time quantitative percent methylation reads and also measure the percent methylation levels of all individual CpG sites in a target region. Determination of two CpG sites of the POMC promoter region by pyrosequencing assay showed that increased methylation of this gene occurred in both FAS and alcohol exposed male and female subjects as compared to control subjects. These data provide first evidence that fetal alcohol exposure makes an epigenetic mark on PER2 and POMC genes in human patients.

The patient cohorts we used to obtain the salivary samples were either born from mothers who abused alcohol and smoked or only abused alcohol. Hence, the possibility arose that nicotine could have influenced the epigenetic marks on PER2 and POMC genes. However, we found that PER2 and POMC gene methylation in control or alcohol-exposed children born from mothers who smoked did not differ from those mothers who did not smoke. The results suggest that maternal smoking did not influence alcohol epigenetic marks on POMC and PER2 genes.

Because the salivary DNA samples have the potential to have bacterial DNA contamination, it is essential to compare the salivary DNA data with blood DNA data. To address this, we have initiated a collaborative study with Dr. Jeffrey Wozniak and his colleagues at the University of Minnesota and obtained peripheral blood mononuclear cell (PBMC) samples from FAS children (age 2 to 3 years) who underwent choline or placebo treatment for 9 months. We obtained PBMC samples at basal, 3 months and 9 months of choline or placebo treatments. We extracted both DNA and RNA from these samples and used them to measure PER2 and POMC gene methylation levels by MS-PCR and PER2 and POMC mRNA levels using RT-PCR. We found that methylation levels of Per2 and POMC genes in PBMC of FAS children of the University of Minnesota are similar to those values in salivary samples of the Indiana University. The patient cohort of University of Minnesota did not have control non-FAS children and thereby we could not compare FAS and control value differences within this cohort. Interestingly however, University of Minnesota patient cohort showed reduction in both POMC and PER2 DNA methylation following choline treatment when the gene expression values of POMC and PER2 were elevated (a similar observation was made by us in the rodent FASD model, Bekdash et al., ACER, 2013). These exciting data demonstrate the known inverse relationship between DNA methylation and gene expression and support the finding obtained from salivary DNA that fetal alcohol exposure makes epigenetic marks on POMC and PER2 genes.

Whether increased POMC and PER2 DNA methylation levels were significantly associated with changes in salivary cortisol levels in fetal alcohol exposed children will be determined by employing salivary samples collected at two CIFASD sites (University of Minnesota and Children Hospital at Los Angles). We are anticipating receiving a good number of samples by mid March. We plan to run cortisol assays in late March and complete the study by early April.

I am also anticipating receiving samples from the Ukrainian patient cohort within a few weeks from Dr. Tina Chambers at UCSD. Additionally, we will be able to determine whether POMC and PER2 gene methylation changes can be detected in mothers during pregnancy who gave birth to FAS children. If higher DNA methylation of POMC and PER2 are alcohol's epigenetic marks originated from mother, they should persist not only in children but also in their mothers at certain stages of pregnancy. Demonstration of such epigenetic marks during pregnancy that persist in FAS offspring should be a significant development to the field, since they will provide the opportunity to identify biomarkers for fetal alcohol exposed patients with the risk of vulnerability for developing neuroendocrine diseases.

Summary: Using state-of-the art epigenetic methods and stringent controls, we have identified epigenetic marks on POMC and PER2 genes in biological fluid samples of FASD patients that persists throughout childhood. Studies are currently ongoing to identify the changes in biological functions associated with the epigenetic modification of these genes. We are also testing whether the epigenetic marks identified in FASD offspring can be detected in their mothers before birth. If successful, this could lead to identification of an epigenetic signature biomarker for FASD patients who are at risk for developing neuroendocrine diseases.

3. For this reporting period (April 1, 2014 - March 1, 2015), is there one or more **Revision/Supplement** associated with this award for which reporting is required?

N/A for Developmental Projects and/or Administrative Supplements

4. What **opportunities for training and professional development** has the project provided?

Nothing to Report

5. How have **results** been **disseminated** to communities of interest? Nothing to Report.

6. What do you **plan to do for the next reporting period** (March 1, 2015 - April 1, 2016) to accomplish the goals?

I plan to continue working with my clinical collaborators to develop and submit a R01 application to determine the changes in biological functions associated with the alcohol epigenetic signatures in FASD children that may lay an important new basis for early diagnosis of an FASD in children and development of novel therapeutic intervention in treating these patients.

7. Describe your project's **interrelation** with aims of the CIFASD consortium its other projects.

We are working with other Consortium projects conducted by Drs. Christina Chambers, Tatiana Foroud, Elizabeth Sowell and Jeffrey Wozniak.

8. **Publications** **[Accepted & In Press]** Nothing to Report.

9. **Publications** **[In Preparation & Submitted]**

We anticipate writing a manuscript soon after completion of our current study.

10. **Poster Abstracts and Presentations** Nothing to Report.

Principal Investigator(s): Tatiana Foroud
Institution(s): Indiana University
CIFASD Project Title: Genomewide SNP Developmental Project
Grant Number: U24 AA014811 (Administrative Core of the CIFASD)

1. What are the **major goals** of the project?

Specific Aim 1: Generate genomewide SNP data for 200 study participants. Individuals with 3D facial imaging and brain imaging will be prioritized.

Specific Aim 2: Utilize the genomewide SNP data to generate principal components reflecting race and ethnicity which can be used to improve the screening tool being developed as part of the facial imaging core.

Specific Aim 3: Test for significant genotype x alcohol exposure effects in key phenotypes (brain imaging, facial imaging, neuropsychological testing).

2. What was **accomplished** under these goals?

Specific Aim 1: Generate genomewide SNP data for 200 study participants. Individuals with 3D facial imaging and brain imaging will be prioritized.

Rationale: Genomewide SNP data will be generated. These data will create a resource for future studies in the consortium. Quality review of all data will be performed and only SNPs and sample performing at a high level will be retained for analysis.

The primary focus in this first year is to generate data for analysis in Year 2. The vast majority of funds have been dedicated for use in genotyping a SNP array in a new set of CIFASD subjects with DNA. These subjects are all from the United States sites. In total, samples from 327 individuals have been sent for genotyping using the new MEGA array which includes over 2 million SNPs and was optimized for samples of various ancestry. The sample included 178 self-reported Caucasian individuals (54.4%), 83 African American (25.4%) and 66 other (20.2%). The sample is nearly equally divided between males and females. Among the 327 individuals, 154 are reported to be exposed to greater than minimal alcohol and 173 were not. Among the 154 who were exposed to alcohol, 24 were classified as FAS, and 130 met criteria for heavy exposure. Among the 173 individuals, 10 had minimal alcohol exposure and 163 were reported to have no alcohol exposure. Previous genotyping was performed using the Illumina OmniExpress array for 236 individuals. When the new data is released, SNP genotyping will be available for 563 individuals. The SNP array data from the 2 arrays can be combined and analyzed together. The SNP data is anticipated to be released in May 2015.

Specific Aim 2: Utilize the genomewide SNP data to generate principal components reflecting race and ethnicity which can be used to improve the screening tool being developed as part of the facial imaging core.

Rationale: Principal component analysis will be used to generate variables that can be used in future analyses. These principal components will serve as quantitative proxies for race and ethnicity in ongoing analyses designed to develop optimal algorithms to identify individuals exposed to alcohol prenatally.

These studies will be initiated once we have the SNP genotypic data.

Specific Aim 3: Test for significant genotype x alcohol exposure effects in key phenotypes (brain imaging, facial imaging, neuropsychological testing).

Rationale: The genotypes generated as part of this project will be used in pilot studies to identify genes and genotypes that interact with prenatal alcohol exposure to predict key study

phenotypes. These phenotypes will include brain imaging measures, principal components generated from facial imaging and neurophysiological measures.

These studies will be initiated once we have the SNP genotypic data.

3. For this reporting period (April 1, 2014 - March 1, 2015), is there one or more **Revision/Supplement** associated with this award for which reporting is required?

Not applicable.

4. What **opportunities for training and professional development** has the project provided?

Ms. Leah Wetherill is a doctoral student in the Department of Psychology and is working on this project. She has been involved in the design of the study and the selection of individuals for SNP genotyping.

5. How have **results** been **disseminated** to communities of interest?

No results have been generated yet.

6. What do you **plan to do for the next reporting period** (March 1, 2015 - April 1, 2016) to accomplish the goals?

Sites for data collection – We continue to collect saliva samples for DNA extraction from all US sites in CIFASD. This developmental project does not collect samples. It only utilizes data and samples generated from other projects.

Data analysis – We anticipate that the SNP array data will be received from CIDR in May 2015. At that time, we will rapidly perform quality review of these data. We will generate principal components that can be used as race/ethnicity specific covariates for analyses. In a sample of mixed race such as has been recruited in CIFASD, the availability of these principal components is essential for subsequent analyses.

Sequencing – We initially explored the opportunity to perform whole exome sequencing in a small number of individuals to explore the potential link between ciliopathy genes and FAS susceptibility. This hypothesis was suggested by analyses in mouse models in the Sulik project. Unfortunately, there are a very large number of genes implicated in the ciliopathies. As a result, we have delayed this hypothesis testing experiment until we have greater data to support the targeting of a smaller number of genes within this pathway.

7. Describe your project's **interrelation** with aims of the CIFASD consortium its other projects.

The Developmental Project has collaborated with the other CIFASD projects to select the individuals for SNP array genotyping. Individuals were selected who were of diverse race and ethnicity and were also selected based on the extent of data available. For example, individuals with data from all domains were prioritized for SNP genotyping. This will ensure that the samples with SNP array data can be used for the widest range of analyses using a diverse series of phenotypic data (neuropsychological, imaging, face shape analyses).

8. **Publications** **[Accepted & In Press]** Nothing to report.

9. **Publications** **[In Preparation & Submitted]** Nothing to report.

10. **Poster Abstracts and Presentations** Nothing to report.

Principal Investigator(s): Peter Hammond[1] and Alison Noble[2]
Institution(s): [1]UCL Institute of Child Health; [2]Institute of Biomedical Engineering, Oxford University.
CIFASD Developmental Project Title: Combining 3D Ultrasound Image Analysis and 3D Facial Morphometry to Detect Craniofacial Effects of Prenatal Alcohol Exposure
Grant Number: U24 AA014811 (Administrative Core of the CIFASD)

1. What are the major goals of the project?

Fetal Alcohol Syndrome (FAS) has a complex phenotype of neurocognitive, behavioural, growth and facial effects. Recognition of the facial characteristics is an essential aspect of the clinical diagnosis of FAS by an experienced dysmorphologist. Currently the facial characteristics are established using objective measures such as head circumference and palpebral fissure width and in addition subjective measures of the upper lip vermillion and philtral groove. A battery of cognitive and behavioural tests are undertaken to evaluate the impact of the prenatal alcohol exposure on the brain. There is substantial evidence that many heavily exposed individuals do not have clinically detectable facial characteristics but do suffer behavioural and cognitive deficits. Consequently, diagnostic accuracy and age of diagnosis remain major difficulties in fetal alcohol spectrum disorders. Earlier diagnosis is becoming more urgent as the efficacy of early intervention has been established in a number of studies. Our ultimate goal is to develop state of the art strategies for prenatal detection of neurofacial effects of *in utero* alcohol exposure using our combined expertise in neural ultrasound and facial shape analysis. For this developmental project, we focused on facial effects with goals as follows:

Goal 1: To develop image analysis algorithms that optimise facial component segmentations in the form of 3D curves and surface patches

Goal 2: To develop pattern matching and machine learning algorithms that detect facial effects of prenatal alcohol exposure in fetuses and neonates

2. What was **accomplished** under these goals?

Goal 1: To develop image analysis algorithms that optimise facial component segmentations in the form of 3D curves and surface patches
Our previous work with 3D photogrammetric images of faces achieved high levels of discrimination between the faces of controls and exposed children using facial dysmorphism detected in the mid-line facial profile (Suttie et al, 2013). Therefore, we began by investigating mid-sagittal profile of the fetal face. With co-operation of the PASS network (in particular, Professor Hein Odendaal of Stellenbosch University), we obtained a small set of 3D ultrasonographic images obtained at 28 weeks gestation. These images were not obtained with a protocol specifically with facial coverage in mind. Therefore, only 21 images were of sufficient quality or provided adequate coverage of the face so that facial components could be segmented successfully. The segmentation of the mid-sagittal profile was achieved using a semi-automated approach summarized below and visualised in figure 1:

1. Use Feature Symmetry (FA) to identify ridges in the ultrasound image (a)
2. Combine Local Energy with Feature Symmetry to find high intensity ridges (a) and segment skull (b).
3. Use orientation of skull to rotate image so that face lies on x-axis (c)
4. Use FA to identify edges in rotated image (d). Consdier only largest connected region where FA is positive.
5. Use Local Orientation (LO) to identify orientation of edges detected in d. e shows regions where LO>0 in green and LO<0 in red
6. Preserve regions with FA response (indicating an edge) and LO greater than 0 (indicating an upwards facing edge) (f).
7. Segment profile (g) - extra segmented example (h)

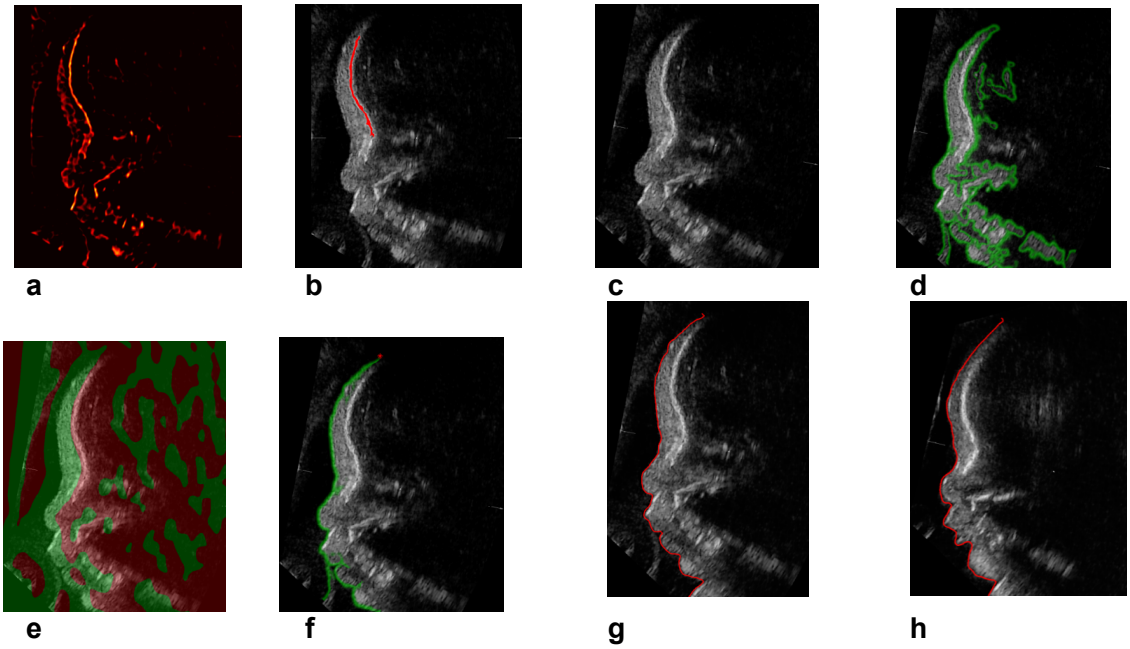


Figure 1 Segmentation of fetal mid-sagittal profile from a 3D ultrasonograph

Although the mid-line facial profile has proven effective in discriminating between the face shape of controls and alcohol exposed children, other face patches are important to test. Therefore, it was essential that we identify the extent of the face surface that is segmentable on a regular basis. By extending the segmentation algorithm described above used to extract the mid-line profile to other orthogonal "slices" of the volume, a series of offset profiles were extracted to form a 3D cloud of points on the fetal face surface.

Figure 2 below shows examples of these point clouds for several fetuses (N.B. because these offset profile curves are in parallel slices they need to be viewed obliquely, otherwise only a series of parallel straight lines are viewable).

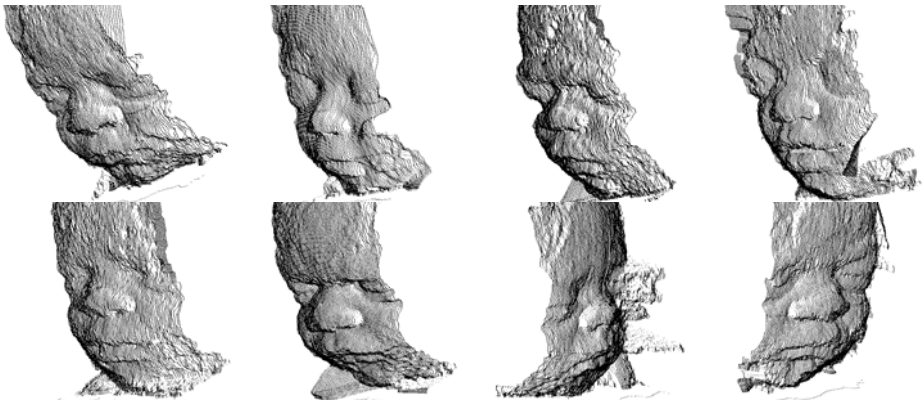


Figure 2 Examples of point clouds formed by the mid-line facial profile and offset profiles
In each point cloud, the philtrum is clearly shown and in most cases the philtral groove or its lack in terms of smoothness is well delineated.

Goal 2: To develop pattern matching and machine learning algorithms that detect facial effects of prenatal alcohol exposure in fetuses and neonates

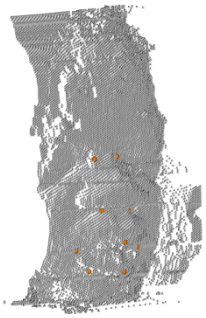
A small number of landmarks was manually placed on the mid-saggital profiles and used to align them automatically to obtain a dense correspondence of points defining the outline profile.

A dense surface model of their shape variation from their mean was then constructed from the set of densely corresponded contours. By re-expressing the contours as a deviation from the mean facial profile principal component modes of variation were identified. The extreme values of the first principal component corresponded to shape variation from a short face with a concave philtrum to a long face with a convex philtrum. The alcohol exposure levels and patterns were not available but collaborators in the PASS network were able to confirm a correlative pattern between this principal component and second trimester alcohol exposure.

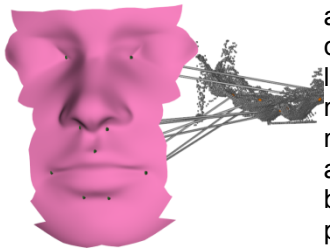
PC1: -2 SD +2SD

Figure 3 Average of 21 segmented mid-sagittal profiles warped to -2 SD and +2 SD values of the first principal component.

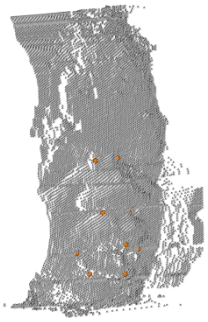
The availability of 3D photos taken at one month and one year of the individuals in the ultrasound images will enable us to make longitudinal comparisons. The 3D photographs are represented as a connected triangular faceted mesh of 3D surface points so conversion of the fetal point clouds described above in C2 will be necessary and likely along the lines of the algorithm outlined below:



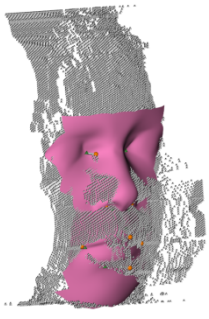
The initial data set consists of the point cloud segmented as described above. The initial surface is a generic face, modified from a stock 3D model, and is defined by a set of mesh nodes.



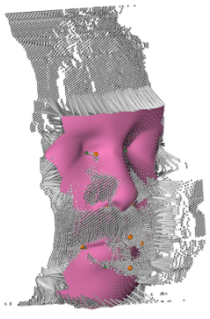
The surface mesh is aligned to the point-cloud pre-defined landmarks. Cost-minimisation drives mesh deformation according to distance between node and point-cloud data



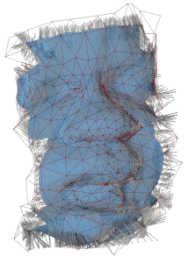
Initial face mesh, marked with nine repeatable landmarks.



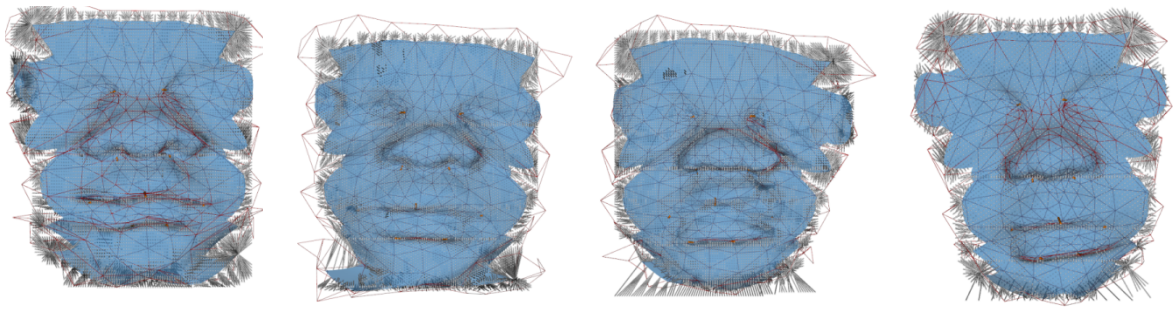
Un-deformed mesh, registered to landmarks.



Initial “driving forces” act on mesh from enclosing points. Each is connected to nearest mesh node.



Final cost-minimised mesh with overlying surface.



Examples of 3D connected meshes derived from the fetal face point clouds

Figure 4: summary of algorithm for converting point clouds to 3D meshes

3. For this reporting period (April 1, 2014 - March 1, 2015), is there one or more **Revision/Supplement** associated with this award for which reporting is required?

N/A for Developmental Projects and/or Administrative Supplements

4. What **opportunities for training and professional development** has the project provided?

Nothing to Report.

5. How have **results** been **disseminated** to communities of interest?

Nothing to Report.

6. What do you **plan to do for the next reporting period** (March 1, 2015 - April 1, 2016) to accomplish the goals?

An R01 is in preparation for prenatal neurofacial analysis of the effects of *in utero* alcohol exposure.

7. Describe your project's **interrelation** with aims of the CIFASD consortium & its other projects.

The aims of this developmental project are in keeping with the CIFASD consortium's aim to develop techniques to support the diagnosis of prenatal alcohol exposure at its earliest opportunity. It draws on the existing facial analysis project supervised by Dr Tatiana Foroud and Dr Peter Hammond. It also will support verification of the mouse studies undertaken by Dr Kathy Sulik's group in terms of the timing effects of exposure.

8. **Publications** **[Accepted & In Press]** None.

9. **Publications** **[In Preparation & Submitted]**

In preparation:

Rackham T, Suttie M, Foroud T, Odendaal H, Hammond P, Noble JA. Prenatal detection of craniofacial effects of *in utero* alcohol exposure: a preliminary study.

10. **Poster Abstracts and Presentations**

Rackham T**,** Fetal face segmentation and contour characterisation in 3D ultrasound**.** Medical Engineering Centres Annual Meeting and Bioengineering14, Imperial College London, 10-11 September 2014.

Principal Investigator(s): Rajesh Miranda
Institution(s): Texas A&M Health Science Center
CIFASD Developmental Project Title: miRNA Profiles
Grant Number: U24 AA014811 (Administrative Core of the CIFASD)

1. What are the **major goals** of the project?

The initial goals of this project were to study circulating miRNAs in biological tissues, specifically in fetal neural stem cells. This goal was revised during the project period, to support the purchase of chemistries and qPCR miRNA arrays, to increase our sample size for plasma miRNA assessments from the Ukraine cohort of pregnant mothers. This is to enable us to complete a sample of ~140 1st and 3rd plasma miRNA profiles from pregnant mothers who have been heavily or moderately exposed to alcohol during pregnancy in Dr. Chamber's U01 project.

2. What was **accomplished** under these goals?

a. Since the receipt of funding, we have been able to place a purchase request for qPCR arrays and associated chemistries from Exiqon, and are awaiting receipt of the supplies. During this project period, we have completed miRNA profiles on 48 plasma samples from the human cohort.

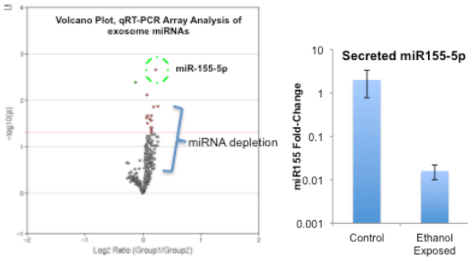


Figure 1 Volcano plot ethanol effects on neuroepithelial cell-derived exosome miRNAs. Ethanol appears to result in depletion of miRNAs from exosomes. miR155 was significantly decreased by ethanol exposure.

b. We have made progress on the original goals of the proposal as well. In this project we focused on miRNAs secreted by fetal neural stem cells in exosomes, small vesicles containing proteins, RNA (miRNAs, mRNAs and other non-coding RNAs) and lipids. Exosomes represent a novel mechanism for cell signaling. We assessed the exosome miRNA contents from control and ethanol pre-exposed mouse fetal neural stem cells cultured as neurospheres. Our data (Figure 1) suggest that ethanol exposure results in a general depletion of miRNAs in exosomes (Figure 1) and that one miRNA, miR155-5p was significantly depleted (by 80-fold) in exosomes released by ethanol-treated neural progenitors. Surprisingly, we also identified a potential target of miR155-5p, the KLF3 mRNA transcript was also co-expressed in exosomes. Intracellular levels of both miR155-5p and KLF3 mRNA were highly positively correlated leading us to a hypothesis that miR155-5p may serve as a chaperone for KLF3 mRNA. KLF3 is an important regulator of stem cell maturation processes in a variety of tissues, and may serve as a transcription repressor. Our working hypothesis is that miR155-5p is a binding partner to KLF3 mRNA transcripts and that this partnership is transferred among neural stem cells as a part of a signaling complex to regulate neural stem cell maturation.

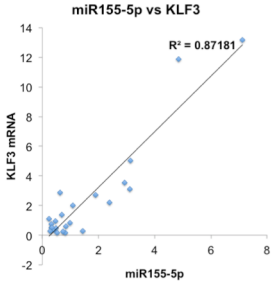


Figure 2 Intracellular expression of miR155 and its mRNA target, KLF3 is highly and significantly correlated.

3. For this reporting period (April 1, 2014 - March 1, 2015), is there one or more **Revision/Supplement** associated with this award for which reporting is required?

N/A for Developmental Projects and/or Administrative Supplements

4. What **opportunities for training and professional development** has the project provided?

This project served as a training program for Dr. Sridevi Balaraman. The Texas A&M Health Science Center, College of Medicine runs a post-doctoral association through the office of the vice-president for research. The association sets up training opportunities like grant writing seminars and career advice seminars for the post-doctoral fellows. Dr. Balaraman is an active participant in these.

5. How have **results** been **disseminated** to communities of interest? Nothing to report.

6. What do you **plan to do for the next reporting period** (March 1, 2015 - April 1, 2016) to accomplish the goals?

We plan to complete the miRNA profiling studies on the Ukraine cohort, using the arrays purchased during this project period. We also plan to continue our functional assessments of circulating miRNAs.

7. Describe your project's **interrelation** with aims of the CIFASD consortium its other projects.

This project has significant interactions with Dr. Chambers project, Early Identification of Affected Children and Risk Factors for FASD in Ukraine (U01AA014835). The focus on function of circulating miRNAs is complementary to the focus on identifying miRNA biomarkers that predict fetal alcohol effect. The purchase of miRNA qPCR arrays will directly assist with increasing the sample size that can be assessed in the Ukraine corhort.

8. **Publications** **[Accepted & In Press]** None.

9. **Publications** **[In Preparation & Submitted]** None.

10. **Poster Abstracts and Presentations**

Balaraman, S., Eaves, S., Skellie, A., Miranda, R.C. Ethanol Sensitivity of Exosome-Shuttled Circulating MiRNAs Released By Fetal Neural Stem Cells. Presented at the Texas Research Society on Alcoholism, TX February 2015.

Principal Investigator(s): William K. Barnett
Institution(s): Indiana University
CIFASD Project Title: Informatics Core for the Collaborative Initiative on Fetal Alcohol Spectrum Disorders
Grant Number: 5 U24AA014818-11

1. What are the **major goals** of the project?

Aim 1.1: It 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.

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

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.

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.

2. What was **accomplished** under these goals?

The following specific aims were addressed during this period:

Aim 1.1: It 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:
    - Added admin dates for Tallies% report for Phase III.
    - Improved tallies history chart with the timeline of recorded tally results.
    - Implemented "Show All records" option in all reports.
    - Updated number of months to default to the latest thirteen months.
- The DemGroupClass Report was modified to reflect updated Demographics records for Phase II

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

- The CIFASD monthly data report was modified to provide historical data to the Administrative Core about the progress that has been made regarding uploading data into the Central Repository.

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.

- Modified the CVLT import function for the Neurobehavior Access input tool.
- Implemented updates to duplicate checking for the EEAC Access input tool and the Central Repository to accommodate multiple biomarkers.
- Updated Biospecimen import function for the Preschool Neurobehavior Access input tool.

- Identified, enforced standardized formatting and corrected erroneous Global ID format of the previously defined Preschool Neurobehavior data based on users' request.
- Created Central Repository for Follow-up/Outcome, Ultrasound and Screener datasets; continue working on the web mechanism to upload and download data to and from the Central Repository.
- Applied additional variables and updated data format for the Eating Habits data dictionary and Access input tool.
- Updated input mask for the Global ID variable in the Dysmorphology Access input tool.
- Created XML upload functionality and discrepancies report for the 2nd Interview (Follow-up/Outcome), Ultrasound, and Screener Access input tools.
- Provided DemGroupClass, Dysmorphology, Brain and 3D Face Imaging tables in relation to Neurobehavior Phase data by implementing a multi-table query.
- Implemented 'Save Report' option to assist researchers in efficient retrieval of the previous data queries.
- Improved identification process for Exposure, Dysmorphology, Demographics, Brain Imaging, and 3D Facial imaging data gathered during Phases I through III to streamline the multi-table query and to provide future access to the Phase I data for external users.
- Updated depiction of Prenatal Child Exposure, Prenatal Source Exposure, Recruited Child Group to incorporate old and new values from the Dysmorphology and Exposure datasets.

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.

- Analyzed Central Repository and provided detailed information on distribution of Phase I data by projects and sites.
- Performed initial design of the security model for the public cifasd website that will allow CIFASD external researchers to access CIFASD data.
- Established and led the Data Access Committee to develop policies, procedures, and facilities so that investigators external to CIFASD can use CIFASD data to further the goals of CIFASD. Developed a Data Use Policy that was approved by the CIFASD Steering Committee and, working with the Admin Core, created a web page on CIFASD.org to guide external investigators.

3. For this reporting period (April 1, 2014 - March 1, 2015), is there one or more **Revision/Supplement** associated with this award for which reporting is required? No

4. What **opportunities for training and professional development** has the project provided?

Nothing to Report

5. How have **results** been **disseminated** to communities of interest? Yes

Information on accessing CIFASD data was added to http://cifasd.org/data-sharing/.

6. What do you **plan to do for the next reporting period** (March 1, 2015 - April 1, 2016) to accomplish the goals?

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

- Continue providing maintenance, changes, and enhancements to various software tools, particularly Phase III data.
- Provide custom datasets, as requested.

- Design and implement secure data access to Phase I dataset for researchers outside of CIFASD through public cifasd website.
- Finalize upload/download Central Repository mechanisms for the Follow-up/Outcome, Ultrasound, and Screener datasets.
- Provide the date of the last data upload in relation to specific datasets on the cifasd index page.

The Informatics Core will remain responsive to the changing information collection, management, and analysis needs of Consortium projects.

Additionally, the Data Access Committee will complete the Data Use Agreement for external investigators and finalize processes and resources for the delivery of CIFASD data to external investigators.

7. Describe your project's **interrelation** with aims of the CIFASD consortium its other projects.

The Informatics Core is the central informatics support for the CIFASD consortium. All projects that collect research data submit them to the Informatics Core, which documents and describes data collected by CIFASD, creates tools for submitting and accessing data, and maintains those data in a secure environment.

8. **Publications [Accepted & In Press]** None.

9. **Publications [In Preparation & Submitted]** None.

10. **Poster Abstracts and Presentations** None.

Principal Investigator (s): **Kenneth Lyons Jones**
Institution (s): **University of California, San Diego**
CIFASD Project Title: **Dysmorphology Core**
Grant Number: **5 U24 AA014815-11**

1. What are the major goals of this project?

Aim #1: To assure consistency as well as accuracy in recognition of Fetal Alcohol Spectrum Disorders (FASD) to all CIFASD project sites throughout the world.
Aim #2: To develop a training DVD that could be sued to teach physicians and other health care professional 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).

2. What was accomplished under these goals?

Aim #1: Overall the Dysmorphology Core has performed 3780 standardized physical examinations on 2615 unique subjects at consortium sites throughout the world. 202 of those children have been examined since April 1, 2014. That included 44 children in Atlanta; 29 children in Los Angeles; 94 children in Minneapolis; 29 children in San Diego; 3 children in Khmelnytsky and 3 children in Rivne in Ukraine.

Aim #2: I have developed three 15 minute DVD of children with the fetal alcohol syndrome that focuses on minor malformations in structural development characteristic of FAS. This careful examination focuses on minor malformations in structural development characteristic of FAS. This can be used on a recurring basis in teaching and clinical practice as a diagnostic tool.

Aim #3: We had initially planned to develop remote sites in two cities in Ukraine. However, following discussions about various alternative strategies necessitated by the need to adapt to various geographic locals, government agencies, individuals and population densities in Ukraine, we concluded that collaboration on this specific aim with one of the Ukrainian sites would not be possible and that collaboration with the other site would be extremely difficult, We therefore established a collaboration with Dr Jeffrey Wozniak at the University of Minnesota to carry out this specific aim. The University of Minnesota has sophisticated previously established telemedicine services that use existing internet and network infrastructure. Over the last year, we created a two phase approach to developing a long-distance consultation service. Through our grant we provided a hand-held camera for taking images. In Phase I, a physician at the University of Minnesota filmed her examination of a child who had been diagnosed as having FAS. The film was then securely transmitted via the internet to me in San Diego for review and a consultation was scheduled. For the consultation, the physician at the University of Minnesota (the remote site) looks at the participant documents and video at her site and I look at the same information on the secure repository at UCSD. I then provide feedback on captured images, text and the consultation is completed with the remote physician using secure imaging software. Following demonstration of our ability to successfully provide a long-distance consultation using this methodology, we will now move to Phase II which will involve real-time consultation (see Section 6: Plans for next reporting period).

Aim #4: circumstances in Ukraine have made this specific aim difficult to carry out. We are not able to collect information in the project timeframe to complete this aim.

3. **For this reporting period (April 1, 2014-March 1, 2015), is there one or more Revision/Supplement associated with this award for which reporting is required?** No.

4. **What opportunities for training and professional development has the project produced?**

At all sites that children are examined by a member of the Dysmorphology Core, a great deal of time and energy is spent in hands-on training of physicians regarding the features of FAS as well as how to diagnose the disorder. This is done with physicians who have no experience with diagnosis of the disorder as well as in re-training physicians who we have previously trained.

In addition, I have begun working with 2nd and 3rd year pediatric residents at UCSD who are involved with Specific Aim #2. Residents who rotate through my FASD Clinic are taught by me on a live patient to diagnose FAS. Their ability to diagnose the disorder will be compared to Residents who learn to make the diagnosis from the training DVD.

The dvd's produced to instruct clinicians and support staff on how to film the dysmorphology exam, what to look for when completing the exam, as well as, handbook materials that outline how prepare the participant packet for consultation can be used over and over again.

5. **How have results been disseminated to communities of interest?** Nothing to report.

6. **What do you plan to do for the next reporting period (March 5, 2015 – April, 2016) to accomplish the goals?**

Aim #1: Over the next year, we will continue to see children ascertained at all CIFASD sites.
Aim #2: We will begin to access the effectiveness of the training DVD and will create additional DVDs on children with FAS as well as other children of different ages and of different ethnic groups who do not have the full syndrome , but who are on the spectrum of defects resulting from prenatal alcohol exposure. We will pursue the opportunities to use these materials in other project, teaching, educational awareness and clinical settings. This includes IRB approval and copyright rules.
Aim#3: We will proceed with Phase II of the methodology for long-distance consultation through initiation of real-time consultations at the University of Minnesota. This will involve taking and helping to set up the transportable exam station (TES) equipment at the University of Minnesota in March 2015. Thereafter, clinical and support staff will be trained on equipment utilizing the software and other documentation implemented in Phase I. Physicians will begin performing the clinical examination and consultation with me during live sessions at the clinic site at the university of Minnesota. The portable equipment will be suitable for use at various sites served by the clinical staff working with Dr. Wozniak including sites in more remote areas of the state of Minnesota.
Aim #4: No plans due to inability to collect information that meets reporting standards.

7. **Describe you project's interrelation with aims of the CIFASD consortium its other projects.**

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

8. Publications [Accepted & In Press]

NIH Public Access Compliance	Citation
Complete	Nguyen TT, Glass L, Coles CD, Kable JA, May PA, et al. The clinical utility and specificity of parent report of executive function among children with prenatal alcohol exposure. J Int Neuropsychol Soc. 2014 Aug;20(7):704-16. PubMed PMID: 25033032; NIHMSID: NIHMS620944; PubMed Central PMCID: PMC4228981.
Complete	Ware AL, Glass L, Crocker N, Deweese BN, Coles CD, et al. Effects of prenatal alcohol exposure and attention-deficit/hyperactivity disorder on adaptive functioning. Alcohol Clin Exp Res. 2014 May;38(5):1439-47. PubMed PMID: 24655090; NIHMSID: NIHMS567036; PubMed Central PMCID: PMC3999227.

9. Publications [In Preparation & Submitted]

Montag AC, Brodine SK, Alcaraz JE, Clapp JD, Allison MA, Calac DJ, Hull AD, Gorman JR, **Jones KL**, Chambers CD. Preventing alcohol-exposed pregnancy among American Indian/Alaska Native population: effect of a screening, brief intervention, and referral to treatment intervention. *Alcohol Clin Exp Res*. 2015 Jan;39(1):126-35. doi: 10.1111/acer.12607

Coles CD, Chambers CD, Kable JA, Keen CL, Yevtushok L, Zymak-Zakutnya N, Wertelecki W, Uriu-Adams JY, **Jones KL** and the CIFASD. Dose and timing of prenatal alcohol exposure and maternal nutritional supplements: developmental effects on 6-month-old infants. *Maternal and Child Health,* submitted August, 2014. CIFASD manuscript submission template completed February, 2015.

Kable JA, Coles CD, Keen CL, Uriu-Adams CD, **Jones KL**, Yevtushok L, Kulikovsky Y, Wertelecki W, Chambers CD and CIFASD. The impact of micronutrient supplementation in alcohol-exposed pregnancies on information processing skills in Ukrainian infants. *Alcohol,* submitted February, 2015. CIFASD manuscript submission template completed February, 2015.

Manuscript in preparation:
Jones KL, Chan PR, Yevtushok L, Zymak-Zakutnya N, Wertelecki W, Keen CL, Chambers, CD. Mechanism involved in variable craniofacial phenotypes following parental alcohol exposure: From mouse to Human. CIFASD concept proposal template completed February, 2015.

10. Poster Abstracts and Presentations

Jones KL, Chan PR, Yevtushok L, Zymak-Zakutnya N, Wertelecki W, Keen CL, Chambers CD. Mechanism involved in variable craniofacial phenotypes following parental alcohol exposure: From mouse to Human. *Alcohol Clin Exp Res*. Jun 2014;38(6):253A.

Coles CD, Chambers CD, Keen CL, Yevtushok L, Zymak-Zakutnya N, Wertelecki W, Uriu-Adams JY, Kable JA, **Jones KL**, and the CIFASD. Preventing effects of prenatal alcohol exposure with maternal nutritional supplements: developmental effects on 6-month-old Ukrainian infants. Presented at the Third European Conference on FASD; Oct 20-22, 2014; Rome, Italy.

Kable JA, Coles CD, Chambers CD, Keen CL, Uriu-Adams J, **Jones KL,** Yevtushok L, Kulikovsky Y, Wertelecki W, and the CIFASD. The impact of micronutrient supplementation in alcohol-exposed pregnancies on information processing skills in Ukrainian infants. Presented at the Third European Conference on FASD; 2014 Oct 20-22, 2014; Rome, Italy.

Chambers CD, Coles CD, Kable JA, Keen CL, Uriu-Adams JY, **Jones KL**, Yevtushok L, Zymak-Zakutnya N, Wertelecki W, and the CIFASD.The impact of micronutrient supplementation in alcohol-exposed pregnancies on gestational age and birth size. Presented at the Third European Conference on FASD; Oct 20-22, 2014; Rome, Italy.

Principal Investigator(s): Christina Chambers
Institution(s): University of California San Diego, La Jolla, CA
CIFASD Project Title: Early Identification of Affected Children and Risk Factors for FASD in Ukraine
Grant Number: 5 U01AA014835-11

1. What are the **major goals** of the project?

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 sheep 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.

2. What was **accomplished** under these goals?

Aims 1 and 2. Child growth and performance:

The primary endpoint for completion of Aims 1 and 2 is the preschool neurocognitive testing performance. The total target sample size for these aims is 240 children tested by the end of the project period. Our cumulative goal for the end of Year 3 (summer of 2015) was 160 children with testing batteries completed. We have completed 116 of these as of February, 2015, as shown in Table 1. We anticipate reaching the target of 160 in 2015, and anticipate being able to conduct a preliminary analysis in 2015 with approximately 2/3 of the final projected sample.

We note that the primary reason for recruiting new pregnant women in Phase III was to provide additional subjects who meet the alcohol exposure criteria but have not had the multivitamin/mineral intervention to ensure sufficient sample size is available to achieve all Aims. The original 5 year goal was 120 women; however, 162 have been recruited in <3 years. All of these women with live born infants will be eligible in the Phase III project to participate in the 2 year old evaluations, and at least half will be eligible for the 3.5 year old preschool testing battery.

Table 1. Recruitment numbers

Type of Data	N Completed Phase III	Type of Data	N Completed Phase III
New pregnant women enrolled	162	3D images	160
2 year old evaluations (growth and development)	117	Blood samples	125
3.5-4.5 year old evaluations (growth, preschool testing battery)	116	Cheek swabs	162
Physiology evaluations (heart rate)	104	Urine samples	158

With respect to growth and eating habits, we have collected growth measures and history of illness for 117 two-year olds and 116 four-year olds as of the date of this progress report. A

preliminary analysis of the Children's Eating Behavior Questionnaire (CEBQ) was completed using data from 86 two-year olds and 55 four-year olds. Food responsiveness scores were on average higher in alcohol-exposed two-year olds compared to low/unexposed (2.3±0.8 vs. 2.1±0.7, respectively); and were significantly elevated in alcohol-exposed four-year olds compared to low/unexposed (2.4±0.6 vs. 1.9±0.6, p <0.0001). In contrast eating rate was significantly slower in both two- and four-year olds prenatally exposed to alcohol compared to similar aged children with low or no exposure (p's<0.05).

Biological samples were shipped in December, 2014: blood and urine to UC Davis and cheek swabs to UCSD. Aliquots of samples archived at Davis were sent to UCSD in support of Aim 4, and to extract DNA for the Developmental Project of Dipak Sarkar.

We have published in this project year on the prevalence and predictors of alcohol use in the first 12,000 pregnant Ukrainian women screened for this study, demonstrating that 46% of the women sampled continued to consume alcohol into mid-pregnancy. We have continued analyses and have two manuscripts under review relevant to the effects of the multivitamin/mineral with or without choline supplement trial. These papers' main findings were:

1. Performance on the Bayley Scales of Infant Development II among six-month old infants by prenatal alcohol dose and by prenatal multivitamin/mineral supplement intervention:
    a. Mental Developmental Index (MDI) scores on the Bayley Scales of Infant Development II at six months of age were significantly impacted by maternal alcohol dose ($X^2_{(1)}$=12.64,p<.000) with more alcohol associated with lower scores and males more affected than females ($X^2_{(3)}$=13.39, p<.004).
    b. Multivitamin/mineral supplementation, as assigned in the trial, had a protective effect among the alcohol exposed; those receiving supplements performed better on the MDI ($X^2_{(1)}$=4.04, p<.04).
    c. The Psychomotor Developmental Index scores did not differ by multivitamin/mineral supplementation assignment but were affected by alcohol dose (β=-26.99, $X^2_{(1)}$ =8.28, p<.004).

2. Cardiac orienting responses to visual and auditory stimuli among six-month old infants by prenatal alcohol exposure group, by prenatal choline supplementation, and by maternal serum levels of choline and metabolites in mid- and late pregnancy:
    a. Choline supplementation, as assigned within the trial in combination with multivitamin/mineral supplementation, resulted in a greater change in heart rate on the visual habituation (Wald Chi-Square (1,149) = 10.9, p < .001, eta-squared = .043) trials for all infants regardless of alcohol exposure group.
    b. The latency of the response was reduced in both conditions (Habituation: Wald Chi-Square (1, 150) = 9.0, p < .003, eta-squared = .056; Dishabituation: Wald Chi-Square (1, 137) =4.9, p < .027, eta-squared = .032) for all infants.
    c. Change in maternal choline level from enrollment to third trimester was positively related (r = .19) to change in heart rate during habituation trials and levels of one choline metabolite, dimethylglycine (DMG), predicted change in heart rate during habituation trials (r = .23) and speed of responses (r = -.20).
    d. There were no significant effects on cardiac orienting responses to the auditory stimuli.

In summary, the beneficial effects of the multivitamin/mineral supplement were seen in the alcohol-exposed group only and only on the MDI in six-month old infants. The beneficial effects of choline in the cardiac orienting response trials were seen in all infants regardless of alcohol

exposure but only in response to the visual stimuli. Changes in maternal serum levels of choline and DMG from enrollment to third trimester were also positively associated with some responses.

Three additional analyses are underway or have been completed. They are summarized as follows:

1. It is hypothesized that some features of fetal alcohol syndrome may overlap with the mild end of the holoprosencephaly spectrum. Both alcohol and retinoic acid have been shown to induce holoprosencephaly-type features in a mouse model dosed at gestational day 7. To evaluate this in the human, we examined maternal Vitamin A levels in mid-trimester among alcohol-exposed and low/unexposed women in relation to selected craniofacial features in the offspring. Infant inner canthal distance was significantly shorter in relation to increasing levels of maternal Vitamin A, consistent with the short inner canthal distance seen with holoprosencephaly; however, alcohol dose as measured in this study did not predict maternal levels of Vitamin A.

2. Alcohol consumption has been shown to increase serum levels of numerous immune cytokines. Elevated maternal levels of IL-6 have been implicated in certain neurological disorders (e.g. autism and schizophrenia) in the offspring. We investigated the hypothesis that IL-6 is a risk factor in alcohol-consuming women who gave birth to a child with FASD. Using third-trimester samples from alcohol-exposed women who subsequently had children with FASD, we found significantly higher plasma IL-6 levels (n=26, -0.22±0.07 log pg/mL) compared to women exposed to similar amounts of alcohol who had children with no features of FASD (n=25, -0.48±0.07 log pg/mL, p=0.007). These data suggest that IL-6 may contribute to or be a marker for risk of FASD in alcohol-exposed pregnancies.

3. Infant iron deficiency has been associated with additional risk for poor outcomes in children with prenatal alcohol exposure. Maternal iron deficiency is also thought to be a risk for poor infant outcomes. Iron status may be altered by alcohol consumption, and could also be impacted by inflammation. We examined serum iron, ferritin, and transferrin receptor measured mid-trimester in 89 alcohol-exposed and 93 low/no exposed pregnant women in relation to birth weight, birth length and birth head circumference of the offspring. Stratifying on multivitamin use during pregnancy, we found no significant effect of any of the iron status measures on any of the growth measures among the unexposed, regardless of vitamin use. However, in the alcohol-exposed group, we noted significant reductions in birth weight, length and head circumference percentiles in association with higher serum iron levels, only among vitamin users (p's <0.02). We also noted significantly lower birth weight and length percentiles with higher serum ferritin measures, again only among vitamin users (p's <0.01).

Aim 3. miRNA:

For the first part of this Aim, three groups of maternal samples were selected from the pool of women with two in-pregnancy blood samples, and physical and neurobehavioral follow-up for their child to 12 months of age: a) women with moderate to heavy alcohol exposure who had a child with FASD, b) women with similar levels of prenatal alcohol exposure who had a child with no features of FASD (normal growth, no facial features, normal Bayley scores), and c) women with low or no alcohol exposure and a child without FASD. We have now completed miRNA profiles on 48 of the selected maternal plasma samples. The following comments are based on analysis of the 1st 36 samples (18 each obtained in mid and late pregnancy distributed equally among alcohol-exposure groups and recruitment sites). There were no significant differences in

erythrocyte contamination between recruitment site and exposure group (all p values>0.46). However, there was a marginal effect of pregnancy stage ($p<0.054$) that needs to be monitored. Late stage pregnancy samples exhibited slightly less erythrocyte contamination, possibly indicative of overall mild anemia. Reliability studies were conducted on a single patient sample (due to cost). This was good evidence for reliability but we would like to replicate a few more patient samples in the next project period to verify measures of reliability. Preliminary analysis indicated that there are group differences in numbers of expressed miRNAs ($F_{(2,24)}=5.32$, $p<0.012$), with one of the three groups expressing the fewest miRNAs. There was also a significant recruitment site difference ($F_{(1,24)}=12.64, p<0.002$), with samples from the Rivne site expressing fewer miRNAs. There was no effect of pregnancy state on miRNA expression. Finally, there were group differences in the average miRNA expression level ($F_{(2,24)}=6.02, p<0.008$), with the same group that expressed the fewest miRNAs exhibiting the lowest miRNA expression levels. Therefore, as the data currently, stands, one group of the three as a whole not only expresses fewer miRNAs, but the plasma expression levels are overall lower. We are in the process of finishing data extraction for the next 12 samples and are starting analysis of miRNA profiles with the next set of 12 samples to make a total of 60 samples (5 samples in each cell for exposure group x recruitment site x pregnancy stage). Additionally, with funds from CIFASD we have also initiated a purchase of additional miRNA qPCR arrays to be able to accomplish miRNA profiling of all 140 samples. We anticipate completing this in the next project period and initiating the analysis of the child samples paired to these maternal samples.

Aim 4. Explore genetic and epigenetic risk factors for FASD, 3D facial images, telemedicine:

We have completed the exploratory epigenetic analysis of maternal samples in this project period. DNA was extracted from buffy coats for 95 samples, and DNA methylation status for more than 450,000 CpG sites was interrogated using the Illumina Infinium HumanMethylation450 BeadChip. Comparing mothers of FASD children to alcohol-exposed alcohol-exposed mothers of unaffected children, we found 9 significantly differentially hypomethylated CpG sites associated with 5 genes, using an adjusted p-value of <0.05. Using a less restrictive raw p-value cutoff of <0.001, 1,799 differentially methylated CpGs were identified associated with 1,025 genes. Expanding the comparison group to the combined group of alcohol-consuming and unexposed mothers of unaffected children, 398 significant CpGs were identified using the most restrictive adjusted p-value of <0.05; 396 of the 398 were hypomethylated, associated with 255 genes. Several of these selectively differentiated genes haven been linked to prenatal alcohol exposure in animal models. Functional enrichment analysis revealed significant enrichment of differentially methylated genes in several pathways including cell adhesion, retinoid binding, glucuronidation, and neuron development.

The results have been shared with others in the CIFASD, and specific suggestions for follow-up in the exploratory genetic analyses solicited. We anticipate initiating the same analyses in the child samples paired to these maternal samples in the next project period.

As shown in Table 1, over the course of the project, we have captured 160 3D images, and those uploaded to Indiana University have been reviewed with feedback provided to the staff who are taking the images. The camera was moved from the Khmelnytsky site to the Rivne site in 2014; however, there have been problems with it functioning at all since that move. At the last site visit in October, 2014, we attempted to repair the camera. However, we have been advised by Dr. Foroud that it needs to be replaced. As the camera cannot be shipped into the country safely, Dr. Chambers will carry it into Ukraine in May of 2015 and capture of 3D images is expected to resume.

The telemedicine project under the Dysmorphology Core was relocated to Minnesota based on logistical barriers to accomplishing this in Ukraine.

3. For this reporting period (April 1, 2014 - March 1, 2015), is there one or more **Revision/Supplement** associated with this award for which reporting is required? None

4. What **opportunities for training and professional development** has the project provided?

Four post-doctoral students in this reporting period (Dr. Lauren Weiss, Dr. Annika Montag, and Dr. Kristin Palmsten in Pediatrics, Division of Dysmorphology and Teratology at UCSD and Dr. Sridevi Balaraman at Texas A&M College of Medicine) have been involved in activities supported by this project and have obtained opportunities for training and professional development. All post-doctoral trainees at UCSD have IDPs in place, and these have been used to manage training for these individuals in terms of skill sets acquired and training in methodology. At Texas A&M, the formal IDPs are in process; these will build on the existing VP for Research-led postdoctoral association that provides cross-discipline formal training opportunities for students.

Four pre-doctoral students in this reporting period (Krista Sowell at UC Davis, Department of Nutrition; Diego Mesa in Bioengineering at UCSD; Tanya Nguyen in Clinical Psychology at SDSU; Amanda Gailey, Georgia State Public Health Program) have also obtained opportunities for training and professional development as part of this project.

5. How have **results** been **disseminated** to communities of interest?

In this project period, we have presented results from the study to the Lviv Medical University faculty in Lviv Ukraine, at the EUFASD meeting in Rome, at RSA, and at the Teratology Society meetings. Results have been shared with Indian Health partners in Southern California, and at the 29th San Diego International Conference on Child and Family Maltreatment. We have presented results through the CIFASD/NOFAS webinar series in June of 2014, and presented results to NICHD and Office of Dietary Supplements staff.

6. What do you **plan to do for the next reporting period** (March 1, 2015 - April 1, 2016) to accomplish the goals?

Aims 1 and 2:

- Achieve target goals for preschool testing battery, and conduct preliminary analyses.
- Compare these results to preschool testing in the CIFASD U.S. sample
- Finalize analyses of growth outcomes and eating behavior measures for the cumulative sample to date
- Perform initial analyses on child biological samples

Aim 3:

- Complete miRNA profiles of the complete set of 140 maternal samples and initiate analysis paired child samples, should funding permit

Aim 4:

- Design and implement approach to exploratory genetics pilot
- Initiate the paired child methylation status component
- Deliver and activate the replacement 3D camera and restart collection of images

We remain concerned about the political situation in Ukraine. Despite the fact that it has been difficult to travel within the country and to transfer money to Ukraine, the site PIs have continued

to collect data and have exceeded recruitment goals for new mothers and infants, providing substantial backfill of new participants to ensure sample size requirements can be met. The site PIs have also been able to successfully ship samples to the U.S. as scheduled and in good order. Regular communication regarding quality assurance and completeness of data collection for the neurobehavioral testing is required and will continue.

7. Describe your project's **interrelation** with aims of the CIFASD consortium its other projects.

This project is interrelated with the Dysmorphology Core for the physical examinations of children in Ukraine; is related to the 3D imaging project with serial collection of images of infants up to seven years of age in Ukraine; is related to Dipak Sarkar's developmental project in aims and by provision of samples; is related to the neurobehavioral testing project with comparable preschool testing batteries being collected in Ukraine. We have had some difficulty with obtaining the appropriate samples in a timely fashion for Dr. Sarkar's project, in part due to the scarcity of the remaining quantities of blood samples from the most informative participants in the original cohort. We are in the process of establishing a study-wide inventory of all samples, types, quantities and physical and storage condition locations that will be provided to the CIFASD repository and investigators and updated on a regular basis.

8. **Publications** **[Accepted & In Press]**

Publications Reported for this Reporting Period

NIH Public Access Compliance	Citation
Complete	Chambers CD, Yevtushok L, Zymak-Zakutnya N, Korzhynskyy Y, Ostapchuk L, et al. Prevalence and predictors of maternal alcohol consumption in 2 regions of Ukraine. Alcohol Clin Exp Res. 2014 Apr;38(4):1012-9. PubMed PMID: 24834525; NIHMSID: NIHMS538981; PubMed Central PMCID: PMC4024828.

9. **Publications** **[In Preparation & Submitted]**

Manuscripts submitted

Coles, C.D., Chambers, C.D., Kable, J.A., Keen, C.L., Yevtushok, L., Zymak-Zakutnya, N., Wertelecki, W., Uriu-Adams, J.Y., Jones, K.L. and the CIFASD. Dose and timing of prenatal alcohol exposure and maternal nutritional supplements: developmental effects on 6-month-old infants. Maternal and Child Health, submitted August, 2014. CIFASD manuscript submission template completed February, 2015.

Carlson, C.R., Jr., Uriu-Adams, J.Y., Chambers, C.D., Yevtushok, L., Zymak-Zakutnya, N., Chan, P.H., Wertelecki, W., Keen, C.L. Low vitamin D status in alcohol-exposed and low/unexposed pregnant Ukrainian women. Submitted to Alcohol, July, 2014. CIFASD manuscript submission template completed February, 2015.

Kable, J.A., Coles, C.D., Keen, C.L., Uriu-Adams, C.D., Jones, K.L., Yevtushok, L., Kulikovsky, Y., Wertelecki, W., Chambers, C.D. and the CIFASD. The impact of micronutrient supplementation in alcohol-exposed pregnancies on information processing skills in Ukrainian infants. Alcohol, submitted February, 2015. CIFASD manuscript submission template completed February, 2015.

Manuscripts in preparation

Jones, K.L., Chan, P.R., Yevtushok, L., Zymak-Zakutnya, N., Wertelecki, W., Keen, C.L., Chambers, C.D. Mechanism involved in variable craniofacial phenotypes following parental alcohol exposure: From mouse to Human. CIFASD concept proposal template completed February, 2015.

10. **Poster Abstracts and Presentations**

Abstracts

Jones, K.L., Chan, P.R., Yevtushok, L., Zymak-Zakutnya, N., Wertelecki, W., Keen, C.L., Chambers, C.D. Mechanism involved in variable craniofacial phenotypes following parental alcohol exposure: From mouse to Human. Presented at RSA, Bellevue, June 2014. *Alcohol Clin Exp Res*, 38(6), 253A.

Feldman, H.S., Zellner, J., Rao, S., Kopald, D., Jones, K.L, Chambers, C. Growth percentile webtool. Presented at OTIS/ENTIS International Meeting, Toronto, Canada, September, 2014. *Birth Defects Res Part A,*100(7), 547-8.

Nguyen, T.T., Risbud, R., Chambers, C.D., Thomas, J.D. Dietary nutrient intake is associated with hyperactivity among children with prenatal alcohol exposure. Presented at the Third European Conference on FASD; Oct 20-22, 2014; Rome, Italy.

Coles, C.D., Chambers, C.D., Keen, C.L., Yevtushok, L., Zymak-Zakutnya, N., Wertelecki, W., Uriu-Adams, J.Y., Kable, J.A., Jones, K.L., and the CIFASD. Preventing effects of prenatal alcohol exposure with maternal nutritional supplements: developmental effects on 6-month-old Ukrainian infants. Presented at the Third European Conference on FASD; Oct 20-22, 2014; Rome, Italy.

Kable, J.A., Coles, C.D., Chambers, C.D., Keen, C.L., Uriu-Adams, J,. Jones, K.L., Yevtushok, L., Kulikovsky, Y., Wertelecki, W., and the CIFASD. The impact of micronutrient supplementation in alcohol-exposed pregnancies on information processing skills in Ukrainian infants. Presented at the Third European Conference on FASD; 2014 Oct 20-22, 2014; Rome, Italy.

Chambers, C.D., Coles, C.D., Kable, J.A., Keen, C.L., Uriu-Adams, J.Y., Jones, K.L., Yevtushok, L., Zymak-Zakutnya, N., Wertelecki, W., and the CIFASD. The impact of micronutrient supplementation in alcohol-exposed pregnancies on gestational age and birth size. Presented at the Third European Conference on FASD; Oct 20-22, 2014; Rome, Italy.

Abstracts submitted

Chambers, C.D., Fisch, K., Sasik, R., Yevtushok, L., Zymak-Zakutnya, N., Wertelecki, W., Schaefer, J., Jepsen, K. Differential methylation patterns in pregnant women carrying fetuses subsequently diagnosed with Fetal Alcohol Spectrum disorders. Submitted to RSA 2015 annual meeting, January, 2015. CIFASD concept proposal template completed February, 2015.

Chambers, C.D., Yevtushok, L., Zymak-Zakutnya, N., Wertelecki, W., Scahefer, J., Boutelle, K. Differential eating behavior patterns in children with moderate to heavy prenatal alcohol

exposure. Submitted to RSA 2015 annual meeting, January 2015. CIFASD concept proposal template completed February, 2015.

Sowell, K., Uriu-Adams, J.Y., Graham, J.L., Chambers, C.D., Coles, C.D., Yevtushok, L., Zymak-Zakutnya, N. Wertelecki, W., Keen, C.L. Implications of altered maternal IL-6 concentrations on infant outcomes of children with prenatal alcohol exposure. Submitted to the Teratology Society 2015 annual meeting, February, 2015. CIFASD concept proposal template completed February, 2015.

Presentations

Grand Rounds OB and Pediatrics, Loma Linda University Medical Center, Loma Linda, CA, April 4, 2014.

CREST (Clinical Research Master's Program)-UCSD, four course lectures April 16 and Nov 13, 2014.

Division of Intramural Population Health Research, NIH-NICHD and Office of Dietary Supplements Seminar. "Fetal Alcohol Spectrum Disorders in Ukraine: The Role of Maternal Nutrition," Rockville, MD; Apr 11, 2014.

NOFAS-CIFASD Webinar on nutrition and FASD, June 18, 2014.

Chairperson, Special Lecture (joint with NBTS), Teratology Society 54th Annual Meeting. "Fetal Alcohol Syndrome." Bellevue, WA; July 1, 2014.

Centers for Disease Control and Prevention, Invited Speaker on FASD, Sept 8, 2014.

The Third European Conference on FASD. "The impact of micronutrient supplementation in alcohol-exposed pregnancies on gestational age and birth size." Rome, Italy; Oct 20-22, 2014.

Lecture, UCSD second year medical students, Nov 12, 2014.

Lectures, Skaggs School of Pharmacy and Pharmaceutical Sciences, 2nd and 3rd year graduate students, Fall, 2014.

Lecture, UCSD USP-144 undergraduate course in Public Health, Dec 2, 2014.

The Chadwick Center's Annual San Diego Conference on Child and Family Maltreatment. "Continuing Challenges with Recognition and Treatment for FASD in the United States." San Diego, CA; Jan 27, 2015.

Principal Investigator(s): Tatiana Foroud and Peter Hammond
Institution(s): Indiana University and University College London
CIFASD Project Title: 3D Facial Imaging in FASD
[Craniofacial Dysmorphism & Fetal Alcohol Exposure]
Grant Number: 5 U01 AA014809-11

1. What are the **major goals** of the project?

Aim 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.

Aim 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.

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

Aim 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.

Aim 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.

2. What was **accomplished** under these goals?

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 359 new images and 521 new DNA samples.

Table 1: Summary of data/sample collection through February 1, 2015 (3dMD only)

Site	3D Images (# subjects)[1]	DNA[1]
San Diego[2]	416 (312)	421
UCLA/USC	104 (88)	159
Atlanta	248 (248)	353
Minneapolis	238 (236)	349
Ukraine	159 (95)	0 (no approval)
South Africa (Jacobson)	517 (312)	225[3]
South Africa (May)	37 (37)	0 (no approval)
South Africa (PASS)	2,361 (1,221)	0 (collected as part of parent study)
Totals	**4,080 (2,549)**	**1,507**

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

[2] This project has also facilitated another grant and has obtained images from 435 children participating in a prevalence study in San Diego.

[3] 218 DNA samples also obtained from the mothers and 52 DNA samples obtained from the father. RNA also collected at this site

Specific Aim 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

***Rationale**:*
In the previous study of South African Cape Coloured children, we used face shape and pattern recognition algorithms to induce classification schemes and established strong agreement with clinical diagnoses of FAS and PFAS. The more heterogeneous phenotype of the non-syndromal but heavily exposed (HE) subgroup forced us to employ a clustering technique, signature graph analysis that normalizes face shape and links individuals with similar facial dysmorphism. Signature graph analysis identified half of the HE group as having facial dysmorphism more FAS-like than control-like. These FAS-like facially dysmorphic HE individuals performed less well on psychometric tests than HE individuals who facially were more control-like. We also demonstrated how heat map comparisons of, and animated morphs between, individuals and matched control means revealed facial dysmorphism otherwise overlooked (Suttie et al, 2013).

In the past year, we have attempted to repeat these results for the Cape-Coloured cohort in a CIFASD recruited cohort of Caucasian and Hispanic individuals. Because there are insufficient numbers of African-American individuals with a diagnosis of FAS, less than 10 currently, we have deferred their analysis for the moment.

Data
A large number of new 3D images have been reviewed and used to construct dense surface models of face shape. Table 2 below summarizes the numbers usable for face shape analysis.

Table 2: Summary of images analyzed (as of February 5th 2015)

CIFASD	Caucasian				Hispanic				Caucasian-Hispanic-Other mix			
Site	HC	FAS	HE	UNK	HC	FAS	HE	UNK	HC	FAS	HE	UNK
San Diego	70	14	24	3	35		14		8		3	1
UCLA/USC	14	5	18	3	1	1	4		3		1	
Atlanta	15	6	9	4			1		2		2	
Minneapolis	22	9	23	0	1		3		4		4	
CIFASD totals	**121**	**34**	**74**	**10**	**37**	**1**	**22**	**0**	**17**	**0**	**10**	**1**
Cape Coloured	**69**	**22**[a]	**75**	**0**								

[a] In the Cape Cohort, both FAS (N=22) and PFAS (N=26) categories were used. In CIFASD, we assume there is only FAS.
HC=Healthy Controls; FAS=Fetal Alcohol Syndrome; HE=Heavily Exposed to alcohol prenatally; UNK=UNKnown alcohol exposure

Preliminary results

Agreement of clinical diagnosis and face classification for Cape and Caucasian cohorts
The rate of agreement of face shape based classification and clinical diagnosis was estimated as the mean area under ROC curves of 20 cross validation trials and corresponds to the probability of correctly classifying two individuals, one taken from each of the control/FAS subgroups being compared. Three pattern recognition algorithms (closest mean, linear discriminant analysis and support vector machines) were used to test agreement with clinical categorization. Since the publication of the Cape Coloured cohort results, subsequent analysis suggested extending the localized regions of the face studied to include philtrum (rather than full upper lip) and malar region. The latter arose after noticing (Fig 1) the stronger and differently located mid-facial hypoplasia in the mean Cape FAS face signature compared to that of the Caucasian cohort. Figure 1C shows the malar area of interest delimited by geodesic curves between well defined facial landmarks. So, as well as the whole face, four patches were also considered in isolation: philtrum, malar region, mid-line profile and nose.

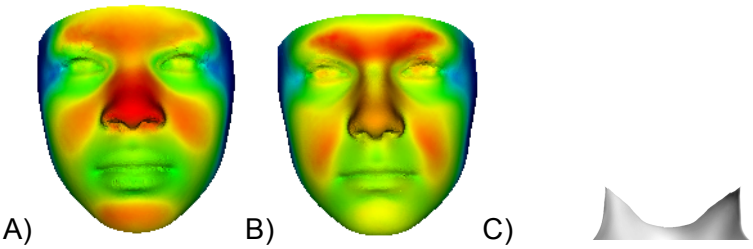


Figure 1: heat map of face signature for depth difference from matched controls for mean of FAS subgroup in Cape cohort (A) and Caucasian cohort (B); malar region of interest (C). Hypoplastic regions are coloured red/yellow.

Agreement between face classification and clinical diagnosis was very strong previously for the full face and mid-line profile for the Cape cohort. Patches focusing on the philtrum and malar only regions were not included in that analysis. When tested recently on the Cape cohort, the philtrum only patch was surprisingly weak and the malar region surprisingly strong in control-FAS discrimination. In contrast, analogous cross-validation testing of agreement with clinical categorization for the Caucasian cohort was much less effective for the full face, mid-line profile and malar region but substantially more effective for the philtrum only patch.

HC vs FAS	**CAPE COLOURED**			**CIFASD CAUCASIAN**		
	CM	**LDA**	**SVM**	**CM**	**LDA**	**SVM**
Profile	0.97	0.97	0.97	0.83	0.85	0.88
Face	0.95	0.95	0.97	0.87	0.88	0.88
Malar	0.95	0.95	0.95	0.79	0.80	0.80
Nose	0.87	0.88	0.90	0.92	0.91	0.94
Philtrum	0.72	0.72	0.78	0.86	0.85	0.86

Table 3 Agreement of face and face patch classification with clinical categorization
CM=closest mean; LDA=linear discriminant analysis; SVM=support vector machines; HC=healthy control; FAS=fetal alcohol syndrome.

These differences in agreement between face only analysis and clinical diagnosis for the two cohorts were much greater than anticipated. Assuming that there is no difference in the diagnostic accuracy of the dysmorphologists who assessed the two cohorts, we needed to consider that the two cohorts may manifest prenatal alcohol exposure effects in different locations facially and to different degrees. The first facial effect we considered further was overall facial growth.

Comparison of facial growth in control/FAS subgroups for the Cape / Caucasian cohorts

The first principal component (PCA1) of a dense surface model of the whole face for a population with a wide age range typically represents overall facial growth. We built separate dense surface models for each cohort so as to avoid the influence of ethnic differences. The overall facial growth of the Cape and Caucasian cohorts is depicted in Fig 2 in terms of PCA1 normalized for age and ethnicity and then z-scored against healthy controls. The much reduced overlap in Fig 2A of the control and FAS subgroups suggests that facial growth reduction might have a stronger role in discriminating between control and FAS for Cape Coloured than for Caucasians. The statistically significant difference in overall facial growth between Cape and Caucasian FAS individuals ($p<10^{-4}$) is much greater than between Cape and Caucasian controls ($p=0.03$). So alcohol exposure appears to have a much greater effect on facial growth in the Cape cohort and since we retain size in our dense surface models this may contribute to better whole face discrimination between control and FAS subgroups in the Cape cohort than in the Caucasian cohort.

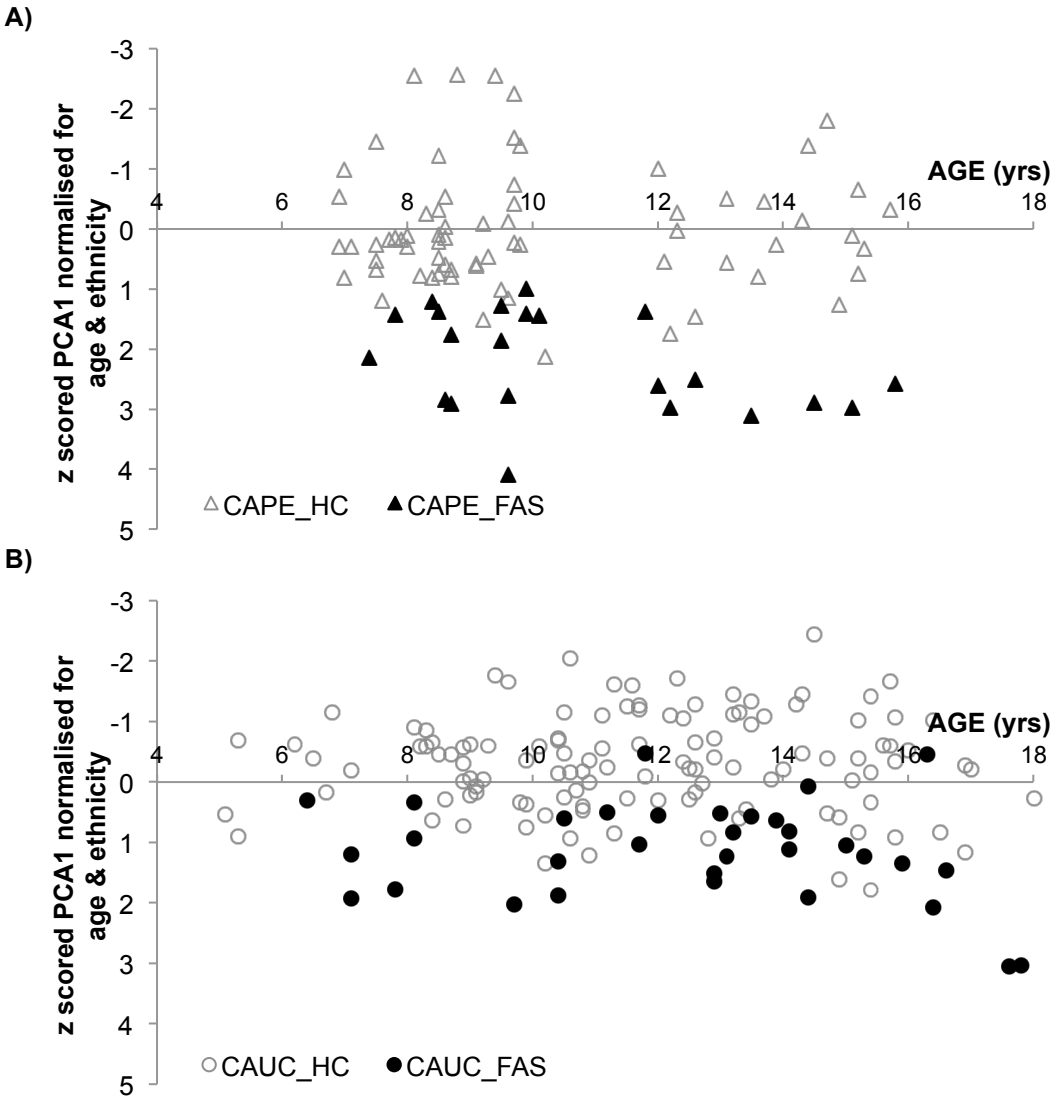


Figure 2: PCA1 for whole face normalized for age and ethnicity normalized and then z-scored
A) Cape Coloured controls and FAS
B) CIFASD Caucasian controls and FAS

Homogeneity of facial dysmorphism of FAS individuals in Cape and Caucasian cohorts
Another potential influence on the agreement of face only classification with clinical diagnosis is the consistency of the alcohol related facial dysmorphism. Because face signature involves normalizing facial dysmorphism against age and ethnicity matched controls, one would expect the ethnic FAS subgroups to mix equally well in a signature graph if they have similar facial dysmorphism.

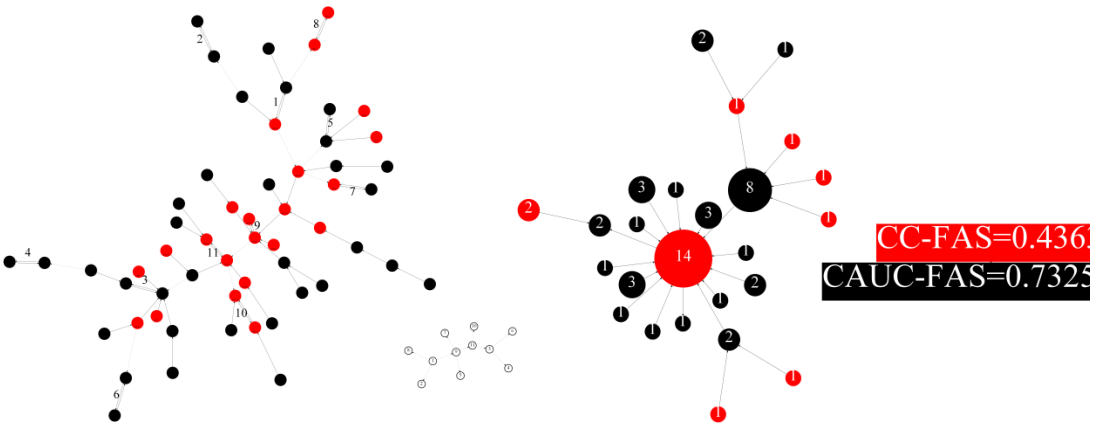


Figure 13
a) Signature graph for whole face for all Cape and Caucasian individuals with FAS;
b) Collapsed form of a) with dispersion coefficients of 0.436 for CAPE FAS and 0.732 for CAUC FAS. Subclusters are made up of individuals with similar facial dysmorphism.

A signature graph for all individuals (Cape and Caucasian) with a clinical diagnosis of FAS (Fig 13A) has a collapsed form (Fig 13B) with dispersion indices of 0.44 for the Cape Coloured FAS subgroup and 0.73 for the Caucasian FAS subgroup respectively. The lower dispersion figure for the Cape cohort reflects the smaller number and/or larger size of FAS subclusters compared to the Caucasian cohort. The corresponding dispersion indices for a signature graph of all Cape and Caucasian controls are respectively 0.78 and 0.72, corresponding to equal ethnic dispersal and hence similar heterogeneity of "dysmorphic" features in controls across both cohorts. These findings suggest that alcohol exposure has had a more homogeneous effect on the Cape cohort than on the CIFASD Caucasian cohort which if true would contribute to better control-FAS discrimination in the Cape cohort than in the Caucasian cohort.

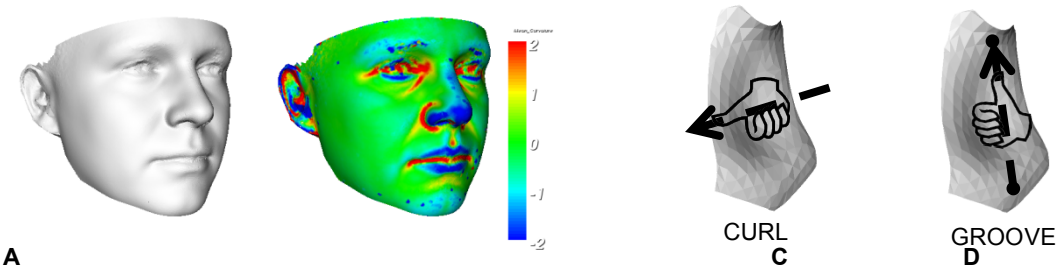
Curvature shape differences in the philtrum of the Cape and Caucasian cohorts

Previously, we have shown that a signature heat map of the philtrum alone revealed a "blue spot" showing the outward expansion of the philtrum groove in the case of FAS. But when the entire face was analyzed, the size reduction in exposed individuals swamped more subtle philtrum differences. One characteristic of face shape that is independent of size is curvature, and therefore we have spent considerable time in the past four months identifying techniques for both the visualization and measurement of curvature of the face surface and in particular the philtrum.

Curvature of the face surface is defined relative to the underlying surface mesh of points which is polyhedral in nature comprising a set of triangular facets. We have altered an algorithm for calculating curvature of a surface that is available in a public library. It determines average curvature at a mesh point in terms of the angles subtended by the surface facets that are adjacent at that point. We soon realized that it is advantageous when focusing on the philtrum to consider two specific curvatures: some individuals have a philtrum that has a deep vertical groove and others have little in the way of a vertical groove but have a curl-like shape (similar to a ski-jump). We use the terminology "groove" and "curl" to distinguish between them and a right hand "rule" as a convenient way to define them (see Figure 3).

The facial landmarks left and right exocanthi are used to define a horizontal axis relative to the face surface and, with the thumb in the direction of the axis (Fig 3C), the fingers follow the curl we are trying to delineate. Analogously, we use an axis defined by the facial landmarks

subnasale and upper lip centre to identify curvature that would form the “vertical” groove of a typical philtrum (Fig 3D).



A **C** **D**

Figure 3

A & B The heat map visualizes average curvature at points across the face surface relative to the scale shown where green indicates no or little curvature at a point, red indicates inward curvature and blue indicates outward curvature. Prominent features such as the nose are blue and grooves or inward creases such as the philtrum are red. Isolated deeply coloured surface triangles are likely to be caused by noise generated during image capture.

C & D A right hand “rule” delineates curvature of the philtrum in a plane orthogonal to the axis along which the thumb points and fingers indicate curve direction; “curl” is used to describe curvature relative to a horizontal axis (defined by the exocanthi) and “groove” is used to describe indentation relative to a “vertical” axis (defined by the line joining subnasale and upper lip centre).

There is considerable variation in philtrum shape in the Cape and Caucasian cohorts. Groove curvature is particularly effective at visualizing the philtral pillars/columns and vertical groove in between (Fig 4).

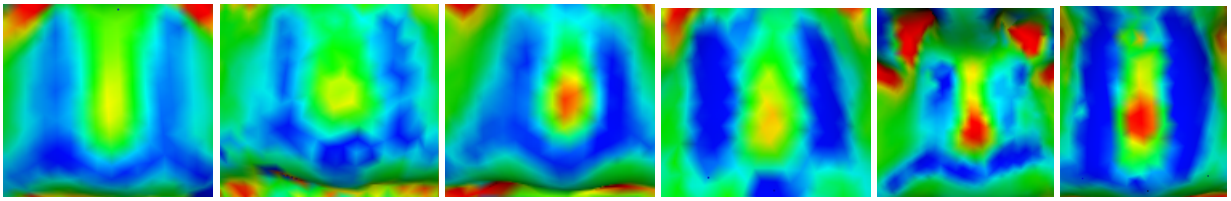


Figure 4 Examples showing visualization of groove curvature of the philtrum. The vertical blue bands highlight the philtral pillars and the red-yellow region the indentation of the philtrum groove.

The independence of curl and groove curvature is demonstrated by the examples below (Fig 5).

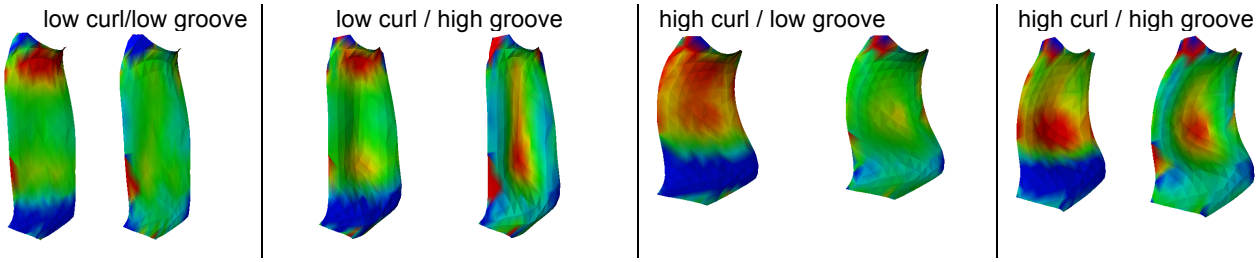


Figure 5 Four examples demonstrating independence of curl and groove in the philtrum

The mean Cape control philtrum has less groove and less discernible philtral pillars close to the nostrils than the mean Caucasian control philtrum. When comparing the Caucasian mean FAS and control philtra, the differences in groove curvature and strength of the philtral pillars are greater than that for the Cape Coloured cohort

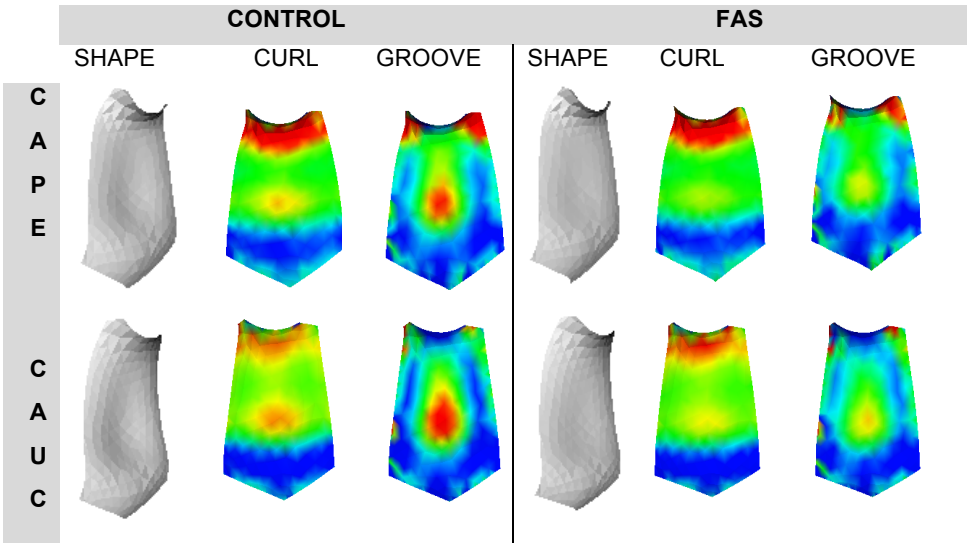


Figure 6 Mean philtrum shape, curl & groove for Cape/Caucasian/control/FAS subgroups

The control (left) half of Figure 6 clearly shows reduced groove and weaker philtral pillars at the upper end of the philtrum in the Cape control mean compared to that of the Caucasian cohort. This identifies greater flatness of the upper philtrum in the Cape controls as an ethnic difference independent of prenatal alcohol exposure. Comparisons of the FAS and control means in the second row of Figure 6 suggest greater control-FAS differences in groove and philtral pillar prominence in the Caucasian cohort compared to the Cape cohort. (N.B. we are currently trying to quantify these differences)

DISCUSSION AND FUTURE DIRECTIONS

Initially, ethnic differences of agreement of face based control-FAS discrimination and clinical diagnosis in the Cape-Coloured and Caucasian cohorts were disappointing. A detailed analysis of facial dysmorphism in the two cohorts has identified potential reasons for these differences. In summary, the facial growth of the Cape FAS subgroup is more significantly reduced than the Caucasian FAS subgroup, and the dysmorphic effects in the former are more homogeneous. These factors likely contribute to the stronger whole face control-FAS discrimination in the Cape cohort. The upper regions of the philtrum in the Cape control subgroup are already smoother when compared to the Caucasian control subgroup and the effects of alcohol exposure may have a stronger philtrum effect in the Caucasian case. The malar region of the Cape cohort appears to be more significantly affected by alcohol exposure than in the Caucasian cohort. These factors could explain the greater control-FAS discrimination in the malar region for the Cape cohort and in the philtrum for the Caucasian cohort. Although the analysis of CIFASD Hispanic and African-American data has been postponed until sufficient individuals with a FAS diagnosis are recruited, it will enhance the Cape-Caucasian comparison if we can delineate differences of dysmorphism in other ethnicities.

A positive outcome of investigating these ethnic differences has been the development of new measures (curl and groove curvature) for visualizing philtrum smoothness and which we believe will also assist evaluation of thinness of the upper lip vermillion. Another bonus is that the curvature techniques can be applied to a raw 3D image without building a dense surface model and with little additional image manipulation on the part of the user. Therefore, they have considerable potential for screening images captured in the clinic. We have produced a draft manuscript describing a face screening tool embodying the techniques described above.

A clinically useful tool for screening the shape of a face using an unprocessed 3D image

The introduction of curl and groove curvature has provided another effective way to delineate face shape variation and in particular has overcome the problem of face size difference dominating more subtle philtrum difference. Therefore, we decided to implement a visualisation tool for inspecting the raw 3D image of a face to aid the clinical evaluation of facial dysmorphism relevant to fetal alcohol spectrum disorders. This enables the raw image to be viewed in 3D, to remove the texture or facial appearance and inspect the philtrum groove, to delineate the groove curvature of the philtrum and to determine the percentile rank of the palpebral fissure length (Fig 7). A detailed description of how to use the tool is provided in a supplementary document. Subject to approval of the CIFASD consortium, we plan to make the tool available for download from the CIFASD website.

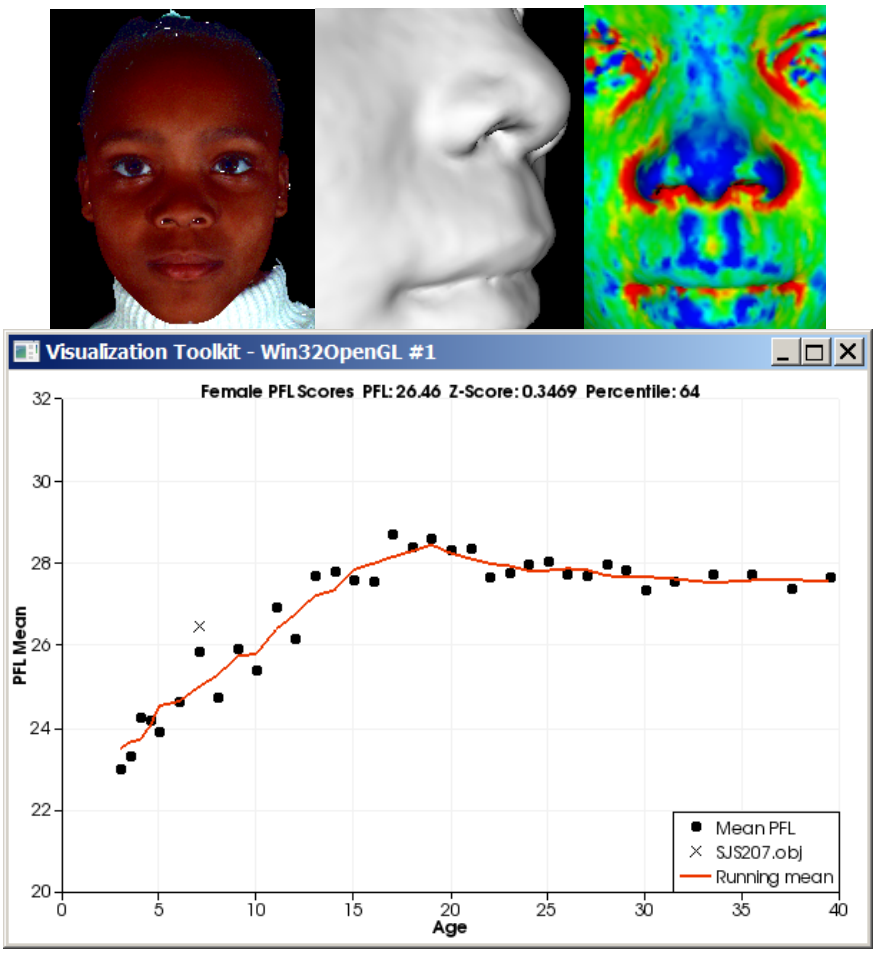


Figure 7 Visualisation of 3D face, surface shape, groove curvature of philtrum and % rank of PFL

The screening tool was first used in the facial evaluation of a young boy, *James,* who is to be one of several children and young adults featured in a one hour documentary *"When pregnant women drink"* to be broadcast by ITV (the prime UK commercial channel) on March 3rd 2015. We haven't seen the programme but at time of writing it has been previewed on ITV news www.itv.com/news/2015-02-24/exposure-when-pregnant-women-drink-the-families-living-with-fasd.

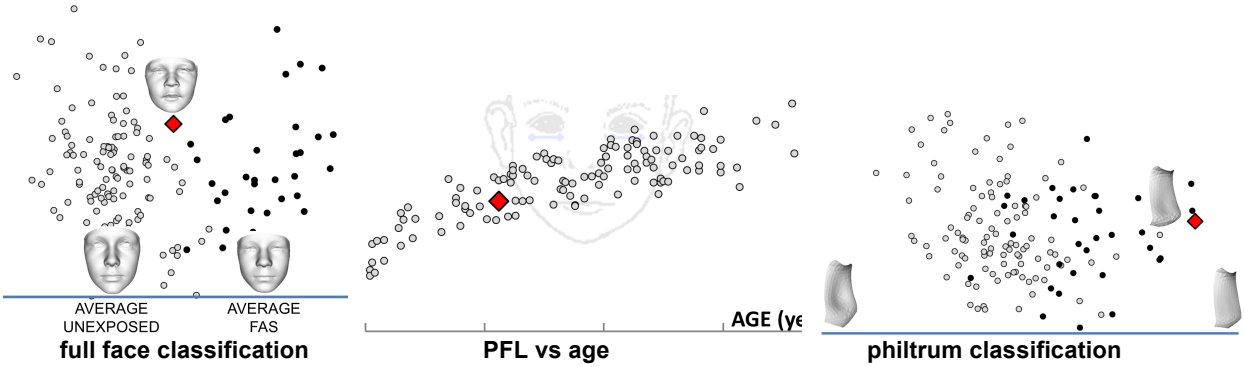
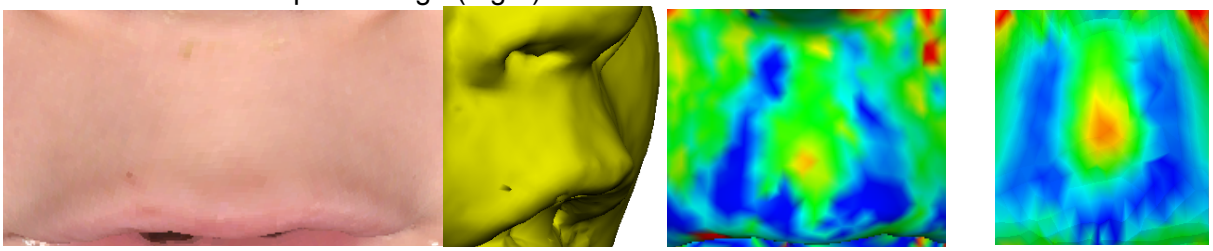


Figure 8 face analysis of *James* employing existing dense surface models of CIFASD subjects

The figure (Fig 8) above shows our usual analysis employing dense surface models involving controls and exposed individuals recruited in CIFASD. It demonstrates that *James* does not have the classical FAS facial features. For his age, his face shape is borderline control-FAS and he has an almost average PFL; but he does have a smooth philtrum and a thin upper lip. The curvature screening tool produced the following comparison of the raw facial image of *James* to a control mean of comparable age (Fig 9).



James philtrum & upper lip | surface shape | *James* groove curvature | control comparison

Figure 9 curvature based analysis of *James' philtrum shape*

A manuscript summarizing our attempt to recapitulate the Cape Coloured face classification results for a CIFASD Caucasian cohort has been drafted. It also includes material described later in this report covering the attempt to recapitulate the prediction of cognitive impairment from facial dysmorphism in heavily exposed individuals without a FAS diagnosis. We are currently discussing whether this manuscript should also include material on the new screening tool or if that should be part of a second manuscript, at a less advanced stage of development, concerned with devising new objective measures of philtral smoothness (and eventually upper lip thinness) described below.

Quantifying philtrum smoothness

We have devised an algorithm for estimating the volume of the philtrum groove by using curl and groove curvature to delineate a region of interest in the philtrum and by constructing a "lid" that would result from the philtrum being "filled up" to the philtral pillars in a tangentially smooth manner (Fig 10). It is quite a challenge to achieve this in an automated manner for every 3D face image given the variation in human philtrum shape, the quality/accuracy/noise of the 3D surface obtained by the 3D camera and the resolution in terms of surface points per sq cm that each camera can acquire. For example, poor camera calibration generates artefacts such as small pyramids and troughs and smoothness may be exaggerated simply because a camera acquires fewer surface points.

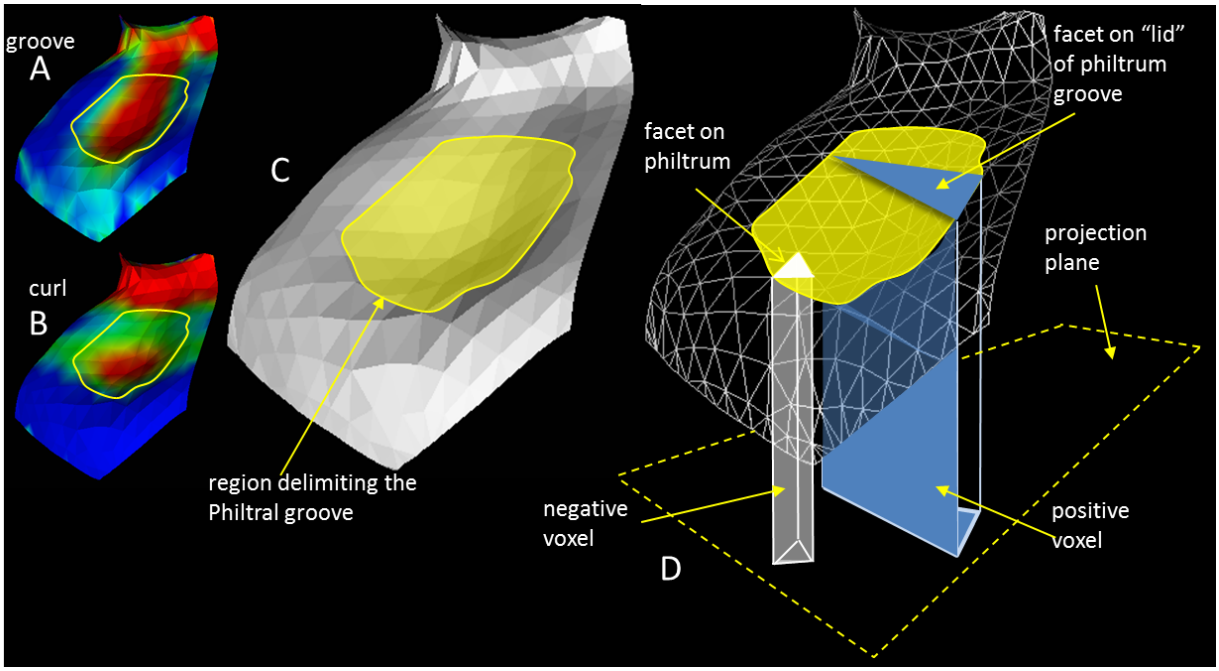


Figure 10 A philtral region is selected using groove curvature (shown blue in ***A***) to produce lateral boundaries of the region of interest - typically coincident with the philtral pillars. Curl curvature (shown blue in ***B***) is used to delimit the lower boundary of the region of interest - typically close to the vermillion border of the upper lip. The upper boundary is identified by a combination of groove and curl curvature. The philtrum region of interest is delineated in yellow in outline in ***A*** and ***B***, and in shaded form in ***C***. Next a "lid" is constructed for the yellow region of interest. Individual triangular facets of the grown region and "lid" are then projected onto an arbitrary plane to produce positive and negative voxels whose net sum is an estimate of the volume of the philtral groove.

We believe that philtrum volume will form the basis for an objective, quantitative measure of philrum smoothness. It will need to be adjusted for age and ethnic background and may need to be converted into linear estimates e.g., average depth of the philtrum. If it produces an effective measure of philtrum smoothness then with the very recent success of the hand held 3D camera in Atlanta in February 2015 its inclusion in the screening tool could support a rapid objective assessment of a face in relation to FASD.

Specific Aim 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.

Rationale:
Based on the results of cross species analyses, we hypothesize that the facial features that distinguish those who are alcohol exposed from those who were not, differ across the lifespan and it may not be possible to develop a single model that can accurately discriminate the groups at all ages. To test this hypothesis, we collecting facial images from 1,200 South Africa PASS subjects at 1 month and 12 months and are using both DSM and face signature analysis methods similar to those in Aim 1. We will compare results in the South Africa cohort with those obtained in the Ukraine sample from which we will obtain images at 6 and 12 months. We anticipate that the novel algorithms developed in this younger cohort will be very valuable in identifying those at high risk for future intervention trials.

Background
Previously some 400 or so 1 month-old and 1 year-old 3D images were kindly provided by the PASS network and also after negotiation approximately 100 were declared as being not alcohol exposed. We generated a list of individuals who showed a smooth philtrum and asked if DM-STAT would compare it to exposure data and provide a sensitivity-specificity summary of exposure-smoothness. DM-STAT's subsequent report showed poor sensitivity and specificity but also suggested rates of philtrum smoothness in controls that were inconsistent with the Cape Coloured cohort described above. As a result, we suggested to the PASS network that we would not complete any further facial analysis until the full exposure data was made available which we expected to be in 2016. However, in mid-November 2014, PASS very kindly provided full exposure data for more than 1,000 individuals. But as other analyses were already under way or scheduled, it has not been possible yet to undertake any analysis apart from some preparatory landmarking of 100 or so of the 600 additional subjects.

Preliminary results N/A

Discussion and future directions
After the FASD conference in Vancouver and the CIFASD annual face-to-face meeting in Rockville in March, we hope to restart the PASS face analysis.

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

Rationale*:*
Based on our initial results, optimal discrimination between controls and those who have been alcohol exposed but do not meet criteria for FAS or PFAS demands the inclusion of non-facial measures. Within CIFASD, data from neurobehavioral testing as well as brain imaging is available and we will develop models that combine specific neurobehavioral measures and 3D facial images to improve our ability to identify those with prenatal alcohol exposure.

Background In the Cape Coloured cohort study, we showed how facial dysmorphism partitioned the heavy exposure (HE) subgroup into FAS/PFAS-like and control-like subsets corresponding to FAS/PFAS-like and control-like cognitive impairment. Because there is no PFAS subcategory in the Caucasian cohort, we retested the original finding but this time omitting the PFAS subcategory. Reassuringly, the HE partition was identical apart from one individual which had no effect on the face-cognitive impairment relationship (Fig 11A).

Preliminary results for face-cognitive impairment analysis
In our first attempt to recapitulate a face-cognitive impairment prediction for an HE subgroup we included both Caucasian and Hispanic individuals. Figure 11B shows a signature graph of 132 alcohol exposed individuals clinically labelled as FAS (red circular border) or HE (green circular or square border). The HE individuals can be separated into two subsets relative to the horizontal dotted line: those above who cluster with the FAS subgroup (green circular border, Fig 11C) and those below who do not (green square border, Fig 11D).

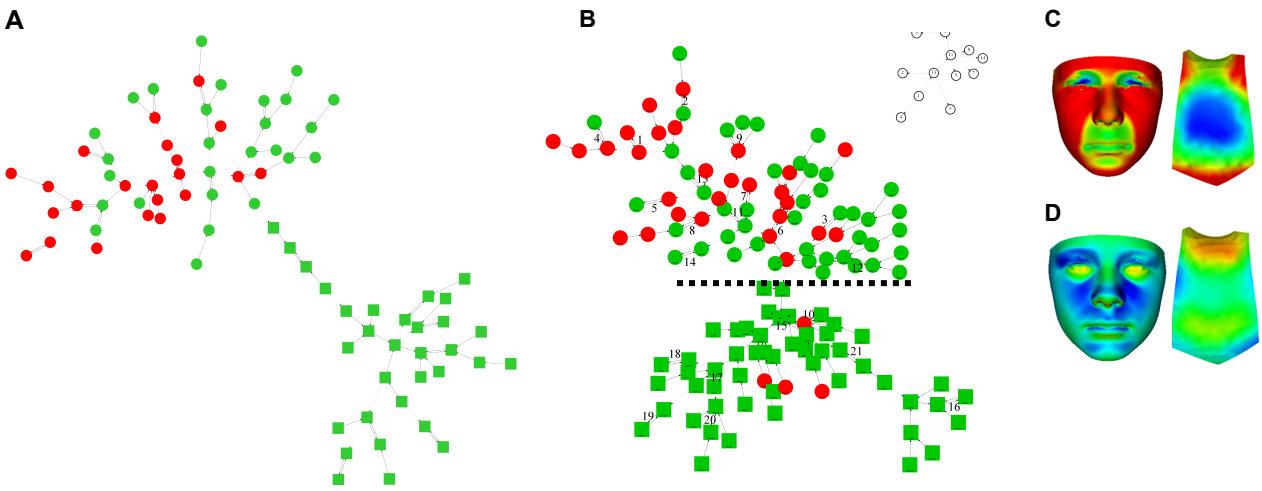


Figure 11 Signature graphs for FAS (red circles), HE who cluster with FAS (green circles) and HE do not (green squares): Cape (A); Caucasian+Hispanic (B); signature heat maps for average Caucasian+Hispanic face and philtrum for HE who cluster with FAS (C) and HE who do not (D).

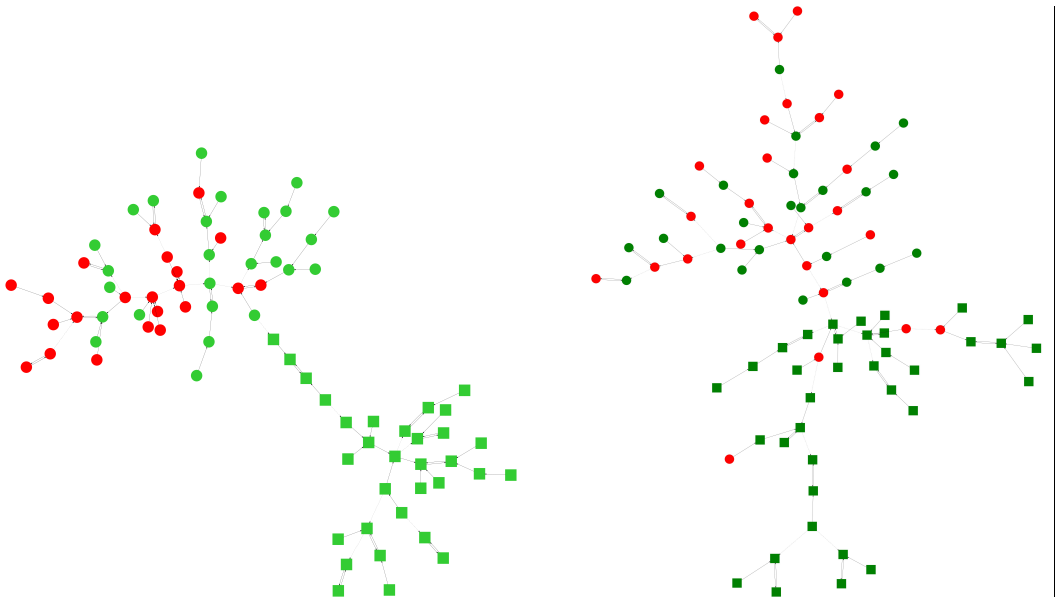
Signature heat maps for means are shown for each HE subdivision (Figs 11C & 11D) displaying face and philtrum dysmorphism at ±1.0 SD and ±0.5 SD respectively. The heat maps for the mean of the FAS-like HE subgroup (Fig 11C) indicates reduced facial growth compared to controls and a smooth philtrum. The heat map for the mean of the remaining HE individuals (Fig 11D) shows slightly increased facial growth compared to matched means and no philtrum smoothness.

The neurocognitive variables shown in table 4 suggest lower cognitive ability for the FAS-like HE subgroup for selected test measures. Scores for CVLT-C verbal comprehension tests are not available for this dataset. However, WISC-IV verbal comprehension and full scale intelligence quotient (FSIQ) variables are available for a subset (n=44) of the HE group. WISC-IV FSIQ scores are marginally lower in the FAS-like HE subgroup although not significantly (p=0.08), a battery of more specific WISC-IV cognitive tests however do appear significantly lower (p<0.05).

	WISC BLOCK SCALE	**WISC MATRREAS SCALE**	**WISC DIGSPAN SCALE**	**WISC COD SCALE**	**WISC FULL SCALE COMP**
HE CONTROL-LIKE	8.81	9.15	8.73	7.19	90.77
HE FAS-LIKE	6.71	7.07	6.86	4.64	79.86
p-Value	**0.05***	**0.05***	**0.05***	**0.02***	0.08

Table 4 Neurocognitive testing measures for HE subgroups in combined Caucasian+Hispanic cohort.

Although these results suggest we can recapitulate the facial dysmorphism-cognitive impairment link previously established in the Cape cohort, after some reflection, the inclusion of Hispanic individuals, only one with a diagnosis of FAS and the rest being controls or HE, was troublesome. This was particularly so given the paper we published in 2014 that showed facial differences between British and Dutch Caucasian individuals involved in another study and our recommendation there that controls should be carefully selected to reflect the affected individuals in any face study. Therefore, we felt it necessary to repeat the analysis without the Hispanic subgroup.



A) CAPE **B) CAUCASIAN**

Figure 12 Signature graphs for FAS (red circles), HE who are FAS-like in facial dysmorphism (green circles) and HE subgroup who are control-like in facial dysmorphism (green squares)

The corresponding signature graph for the combined FAS and HE subgroups of the Caucasian cohort (Fig 12B) does partition the Caucasian HE subgroup although it is less clear cut with 4 FAS individuals not clustering with their FAS peers. But, most importantly, the partition does not correspond to FAS-like and control-like significant difference in cognitive impairment.

Preliminary results for face-brain analysis

This preliminary analysis involved an existing CIFASD dataset (n=116), a subset of the US Caucasian/Hispanic subjects as outlined in objective I, aged 7-17 with varying degrees of alcohol exposure and includes subjects clinically labelled as FAS (n=19), HE (n=46) and unexposed HCs (n=49). The interval between the MRI and 3D face data acquisition is on average 3 months (SD 4.5 months) with those with a period of greater than 9 months between scans excluded from the study. Standardised batteries of neuropsychological tests were administered within the CIFASD consortium including the WISC-IV scores used in our previous study, but with limited coverage in this dataset. Given this lack of consistent WISC-IV measures, equivalent neurocognitive scoring metrics were taken into account to give a full scale intelligence quotient (FSIQ). FSIQ scores are predictably lower in the alcohol exposed subjects (FAS μ=87.5, HE μ=90.6) than in the unexposed controls (HC μ=109.9).

Methods

Prior to the application of combined DSM, images from both modalities require pre-processing. CC midline representations were obtained from a contour tracing of points from the raw MRI scans. Each subject was registered to MNI152 space via FSL's flirt (Jenkinson et al 2012). A midline slice is selected and the trace starts from the posterior aspect of the genu to the inferior-most point of the splenium for both top and bottom segments of the CC (Fig 14A). The points for the upper and lower segments are then concatenated to form a continuous midline CC contour representation (Fig 14B). Equidistant +/- Z points were induced either side of each vertex, edges are drawn from these points to the original contour points to create an artificially triangulated surface mesh. Surface normals are then calculated by taking the vector cross product of the created edges. Three anatomical landmarks were added manually; the lower point of the splenium, the tip of the genu and a midpoint along the upper arch. These points

were used to generate a clipping plane through the midline of the 3D surface. Surface geometry was extracted from the intersecting points within this plane to produce an accurate representation of a midline contour. The resulting contour is used to produce 35 additional equidistant quasi-landmarks between the three original points; these are calculated based on equal geodesic distance determined by the shortest path. The resulting quasi-landmarks (Fig 14C) comprise 10 from genu to mid upper point, 10 from genu to upper and 15 on the inferior arch from the genu to the splenuim.

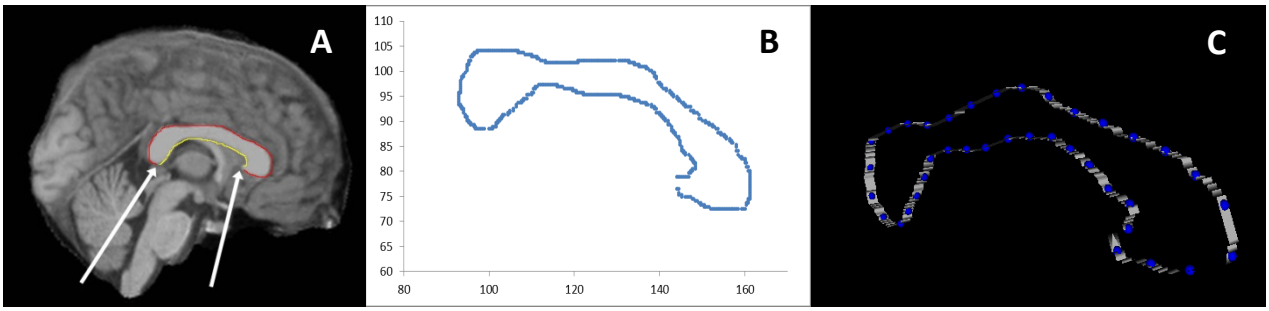


Figure 14 (A) Original contour outline on MRI, splenium and genu points (B) Plot of raw corpus callosum points in 2D from contour outline (C) 38 landmarks including 35 semi-automatically induced equidistant quasi-landmark points

Four twin surface dense surface models were built separately pairing each of the full face, facial profile, peri-orbital region and philtrum with the mid-line contour of the corpus callosum. PCA representations within the model now characterize covariance between the face and brain PCs. In each of the individual face component models, the first mode of variation (PC1) continues to be representative of facial growth (Figure 15A) as in previous DSM face models (Hammond & Suttie, 2012).

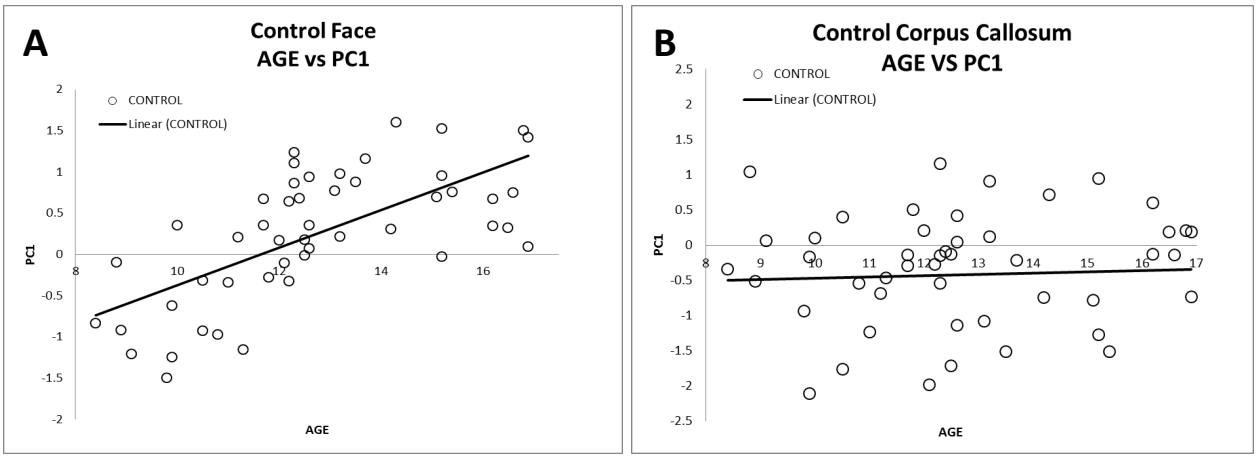


Figure 15 (A) Facial growth represented by the first mode of variance correlating with age (PC1) (B) PC1 showing no correlation with age, diverging from the traditional growth trajectory often seen in face DSM models.

Mean Comparisons - FAS

Mean comparisons of face-brain dysmorphology in combined DSM models correlate with previous findings for both components. Mean philtral smoothness in the FAS group is clearly indicated by a blue spot centred in the philtrum (Figure 16A), whilst in the same model, the CC shows region-specific thinning in both anterior and posterior sections. Equally, face and CC combined DSM representation (Figure 16B), shows significant facial growth delay in FAS individuals. Finer features are harder to discern in this representation as the overpowering influence of size has a detrimental effect on the detection of minor localised shape variance.

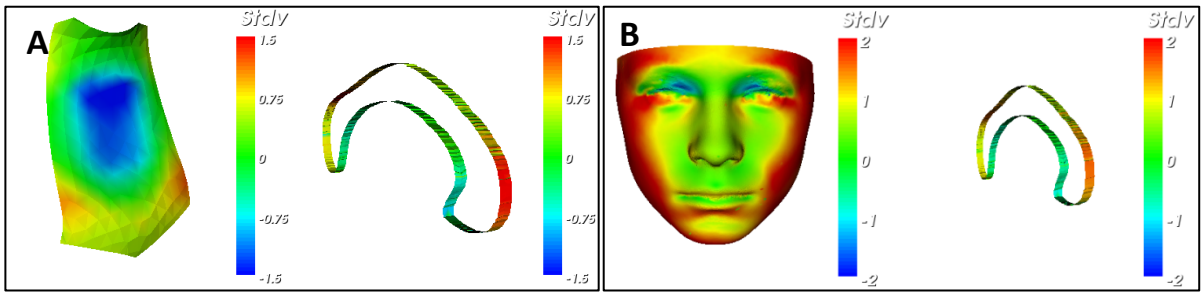


Figure 16 FAS mean (n=19) normalised differences for philtrum + CC at 1.5SD (A) and face + CC at 2SD (B) extracted from a combined DSM model (n=115). Red-green-blue denotes surface retraction-coincidence-expansion compared to an age-matched control mean (n=30)

Discrimination Testing of Face Patches in combination with Corpus Callosum

Once again, we used CM, SVM and LDA algorithms for 19-folded testing of classification of FAS and HC groups using the set of combined face-CC PCA values. A CC only model representation produced a mean overall accuracy of 0.93, showing the CC alone to be highly discriminating. In testing individual face patches alone and in combination with the CC we observed equal or marginally improved results in the combined models with greatest effect for philtrum+CC (Table 5).

	Single DSM	Combined DSM (+CC)
Philtrum	0.95	1.00
Profile	0.95	0.97
Face	0.90	0.90
Peri-orbital	0.95	0.95

Table 5 Discrimination results for FAS+PFAS (n=19) vs HC (n=49). First column depicts classification accuracy for single surface models within this dataset for philtrum, mid-line profile, full face and peri-orbital regions. Second column indicates results obtained from combined modelling discrimination testing with the same facial components and the addition of the corpus callosum. In each case we see classification performance either unchanged by the additional structure or marginally improved.

Preliminary results for Face, Brain and Neurocognitive Profile

Brain morphology and neurocognitive performance in both alcohol exposed and control subjects are known to be related (Riley et al, 2005). This suggested that our combined face-CC DSM models may potentially show association with neurocognitive variables. PCA1 for the combined face-brain DSMs represents the correlation with the largest variance. For the combined philtrum-CC model PCA1 delineates variation in size and smoothness of the philtrum coinciding with anterior and posterior thinning and displacement of the CC. For the Caucasian FAS and HC individuals we see a significant correlation between FS1Q and PCA1 of the joint philtrum-CC model (Fig 17). This is a clear indicator that the morpohological difference expressed by PCA1 variation is correlated with neurocognitive performance.

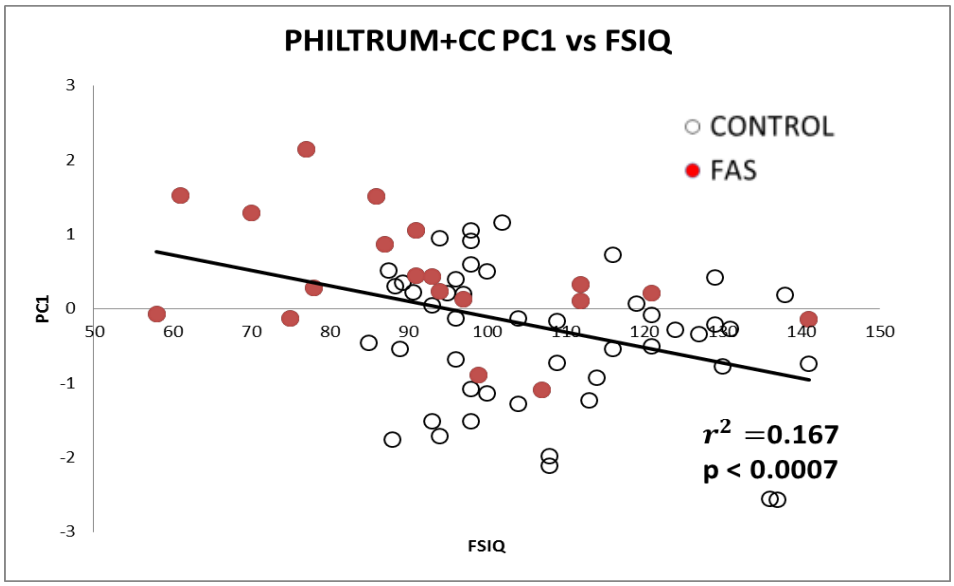


Figure 17 PCA1 for the combined philtrum and corpus callosum model represents philtrum smoothening and length and anterior-posterior thinning and displacement of CC. It is significantly correlated with FSIQ. (P<0.0007)

Discussion

The recapitulation of the Cape face-cognitive impairment partition of HE individuals induced by a signature graph of HE and FSA subgroups was only possible when Hispanic individuals were added to the Caucasian cohort. This may be due to the different facial effects established earlier and proposed as explanation for the inconsistent agreement of face analysis and clinical diagnosis in the two ethnic cohorts. Another issue is that different cognitive batteries of measures were employed at different stages of CIFASD. Some corresponding measures show a consistent difference suggesting they need adjustment before combined use. Finally, we believe that all combined face/psychometric/brain analysis should be improved by the introduction of an objective, quantitative measure of philtrum smoothness.

Specific Aim 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

Rationale: *Existing software tools analyze hundreds of 3D images simultaneously subject to limitations of a 32-bit architecture. The aims above will require analyses of thousands of images to support broader age ranges, integration of non-facial data, ethnic comparison and comparison with developmental delay of a genetic or non-alcohol related origin. We will migrate existing software to a 64-bit architecture and develop new analytical approaches to take advantage of increased computational power.*

Progress

We have completed the software migration and extensions to support the construction of dense surface models of numbers of individuals limited only by the computer RAM available. The new techniques that these improvements enable are dealt with under the earlier aims.

Specific Aim 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.

Rationale:
A goal of CIFASD is to identify those at greatest risk for physical and behavioral abnormalities following prenatal alcohol exposure. Preliminary data suggests that there may be DNA polymorphisms that have differential effects on facial development in the presence or absence of alcohol prenatally. We will continue to explore this hypothesis using candidate genes nominated from other projects in CIFASD.

Data

We have continued to collect saliva sample for DNA extraction from subjects in the United States. Analyses using existing SNP array data was performed to follow-up genes identified in the development project led by Johann Eberhardt as well as the project pursued by Michael Charness. Results from extended analysis of *PDGFRA* and *FYN* were presented at RSA in 2014 and will be presented again at the Vancouver FAS meeting.

SNP GWAS arrays are being genotyped in an additional 300 subjects from the United States. The data from these samples are expected later this spring. These data will allow analyses to be expanded to a larger portion of the CIFASD cohort.

3. For this reporting period (April 1, 2014 - March 1, 2015), is there one or more **Revision/Supplement** associated with this award for which reporting is required? N/A

4. What **opportunities for training and professional development** has the project provided?

Within the project, there are two students who are completing training. Dr. Hammond is the primary mentor for Mr. Michael Suttie, who is completing his doctoral degree at University College, London. Leah Wetherill is also a trainee completing her doctoral degree within the Department of Psychology at IUPUI. Both trainees have had the opportunity to attend conferences and participate in the preparation of manuscripts.

5. How have **results** been **disseminated** to communities of interest? Presentations at relevant conferences.

6. What do you **plan to do for the next reporting period** (March 1, 2015 - April 1, 2016) to accomplish the goals?

Sites for data collection – Data collection will continue at all current sites.

Data analysis – Algorithm development and software implementation – The sections above have outlined the plans for the next year.

7. Describe your project's **interrelation** with aims of the CIFASD consortium its other projects.

Face-Brain Subgroup – Currently, there are 567 individuals with both 3D image and neurocognitive data. There are 136 individuals with both 3D image and brain volume data (MRI). There are 121 individuals with data from all three domains: 3D image, neurocognitive, and brain volume data (MRI). Hypotheses are being developed which will explore the impact of alcohol exposure on these various domains in this growing sample.

We recently participated in a "work-group" including the Neurobehavior, 3D facial imaging, neuroimaging, and genetics projects to integrate neuroimaging findings with those from basic

science. This group has identified candidate genes from zebrafish and human cell models as being associated with susceptibility to the harmful effects of prenatal alcohol exposure. As a group, we determined the most meaningful variables from the various project domains (a priori hypotheses). The preliminary dataset consisted of 10 principal components describing facial shapes, 10 principal components describing the philtrum, 3 executive function tasks and 5 corpus callosum brain volumes. Genetic analyses were conducted to examine if there was a genotype by alcohol exposure interaction; i.e., is the genotype, in the presence of prenatal alcohol exposure, protective of the phenotype (e.g., less dymorphic face, better executive function, increased brain volume). Preliminary results for *FYN*, a member of the Src kinase family, demonstrated moderately significant association of genetic markers in the gene with facial shapes, executive function, and brain volume, in the presence of prenatal alcohol exposure. These findings formed the basis of our upcoming Symposium to be presented at RSA in June, 2015.

Coordination with the Bioinformatics Core – The Bioinformatics Core members have created a new interface for uploading the data so that xml and mdl files can be uploaded simultaneously. The x,y,z, coordinate data are now a part of the data base. The new interface accommodates longitudinal data. The Bioinformatics Core has also improved the rate at which images can be uploaded to the central repository, and is working to implement a method to track in the Central Repository. Images obtained for CIFASD subjects recruited through a CIFASD site are included.

The SNP genotypic data is available in the central repository. In addition, principal components generated from the SNP genotypic data are available for CIFASD investigators to use as race covariates in analysis. A new variable was added to the central repository that indicates if the subject has a DNA sample available.

Coordination with Dysmorphology Core - All subjects were seen by a member of the Dysmorphology Core and assigned a diagnosis of FAS, deferred, or no FAS.

Coordination with mouse project – Mike Suttie has continued to use the atlas-based software provided by UCL colleagues to process a collection of mouse MRI images for collaborators at UNC. When their transfer to Kathy Sulik's team and their segmentation is complete, we will continue the collaborative comparison of neurofacial effects of timing of exposure.

8. Publications [Accepted & In Press]

Hopman SMJ, Merks JHM, **Suttie M**, Hennekam RCM, **Hammond** P. Face shape differs in phylogenetically related populations, *Eur J Hum Gen*, 2014, 22(11):1268-71. doi: 10.1038/ejhg.2013.289. PMID: 24398794. PMCID: PMC4200424.

Hammond P, McKee S, **Suttie M**, Allanson J, Cobben J-M, Maas SM, Quarrell O, Smith ACM, Lewis S, Tassabehji M, Sisodiya S, Mattina T, Hennekam R. Opposite effects on facial morphology due to gene dosage sensitivity, *Hum Gen,* 2014, 133(9):1117-1125. doi: 10.1007/s00439-014-1455-z. PMID: 24889830. PMCID: PMC4148161.

9. Publications [In Preparation & Submitted]

None.

10. Poster Abstracts and Presentations

Hammond P. (Symposium talk) Analysing Genetic and Teratogenic Effects of Alcohol on Facial Form. ISBRA/RSA Joint Congress (37th Annual RSA Scientific Meeting), Bellevue WA USA, Jun 21-25, 2014.

Kodali V, Jacobson J, **Suttie M**, Dodge N, **Wetherill L**, Molteno C, Meintjes E, Hoyme E, Robinson L, Khaole N, **Foroud T**, **Hammond P**, Jacobson S. (abstract 0708) Facial imaging can provide a marker for verbal performance deficits in children with alcohol-related neurodevelopmental disorder. ISBRA/RSA Joint Congress (37th Annual RSA Scientific Meeting), Bellevue WA USA, Jun 21-25, 2014.

Dou X, **Hammond P**, **Suttie M**, **Wetherill L**, **Foroud T**, Chen S-Y, Chen X, Charness ME. (poster) Src Phosphorylation of L1 Modulates Ethanol Toxicity in Developing Nervous System. ISBRA/RSA Joint Congress (37th Annual RSA Scientific Meeting), Bellevue WA USA, Jun 21-25, 2014.

Wetherill L, **Hammond P**, **Suttie M**, Jones K, Coles C, Sowell E, Mattson S, Riley E, Eberhart J, Kou X, Charness ME, **Foroud T**. (symposium) Gene X Prenatal Alcohol Exposure: Preliminary Results in SRC Family Kinase and PDGF Pathways. ISBRA/RSA Joint Congress (37th Annual RSA Scientific Meeting), Bellevue WA USA, Jun 21-25, 2014.

Suttie M, **Wetherill L** Jones K, Coles C, Sowell E, Mattson S, Riley E, **Foroud T**, **Hammond P**. (symposium) Analysing genetic and teratogenic effects of alcohol on facial form. ISBRA/RSA Joint Congress (37th Annual RSA Scientific Meeting), Bellevue WA USA, Jun 21-25, 2014.

Hammond P. (invited talk) Genetic and Teratogenic Effects on Craniofacial Form. 35th Annual David W. Smith Workshop on Malformations and Morphogenesis, Madison WI, Jul 29, 2014.

Hammond P. (Invited talk) Face shape differences due to deletion & duplication of genes typically associated with Williams syndrome. 11th Australian Williams Syndrome Conference, Sydney, Australia, Sep 19-21, 2014.

Hammond P. (Invited talk) 3-D Facial Imaging & Fetal Alcohol Syndrome Disorders. 3rd EUFASD Conference (Third European Conference on Fetal Alcohol Spectrum Disorders), Rome, Italy, Oct 20-22, 2014.

Hammond P. (Symposium talk) Analysing Genetic and Teratogenic Effects of Alcohol on Facial Form. 6th International Conference on FASD, Vancouver, Canada, Mar 5-7, 2015.

Hammond P. (Symposium talk) Facial Clues to the Effects of Prenatal Alcohol Exposure on Brain and Behaviour. American Psychiatric Association 168th Annual Meeting 2015, Toronto, Canada, May 16-20, 2015

Foroud T. (symposium) Gene X Prenatal Alcohol Exposure: Preliminary Results in Src Family Kinase and Pdgf Pathways. 6th International Conference on FASD; Research: Results and Relevance 2015, Vancouver BC Canada, Mar 5-7, 2015.

Suttie M. (presentation) MPhil/PhD Upgrade Presentation, University College London Institute of Child Health, London, UK, Aug 2015

Principal Investigator(s): Elizabeth R. Sowell
Institution(s): Children's Hospital Los Angeles
CIFASD Project Title: Mapping the Brain, the Face and Neurocognitive Function in FASD
Grant Number: 5 U01 AA017122-08

1. What are the **major goals** of the project?

In each of 3 Specific Aims, we will test hypotheses regarding: **a.** cross-sectional and longitudinal differences in brain structure (high-resolution T1- and T2-weighted MRI), structural connectivity (diffusion tensor imaging), and functional connectivity (resting state fMRI), and the relationships between or differences among imaging markers in FASD and unexposed children at 4 sites (Los Angeles, San Diego, Atlanta (new), and Minnesota (new)); **b.** relationships among neurocognitive measures and cross-sectional and longitudinal brain "imaging biomarkers;" **c.** relationships between dysmorphology of the face and the brain, and **d.** how dysmorphology in the brains of children associate with findings in animal models.

Aim 1: To evaluate cross-sectionally and longitudinally the effects of prenatal alcohol exposure on brain morphology and connectivity using high-resolution T1- and T2-wieghted MRI datasets.
Aim 2. To evaluate cross-sectionally and longitudinally the effects of prenatal alcohol exposure on functional connectivity using resting state (rs) fMRI.
Aim 3. To evaluate cross-sectionally and longitudinally the effects of prenatal alcohol exposure on structural connectivity using diffusion tensor imaging (DTI).

2. What was **accomplished** under these goals?

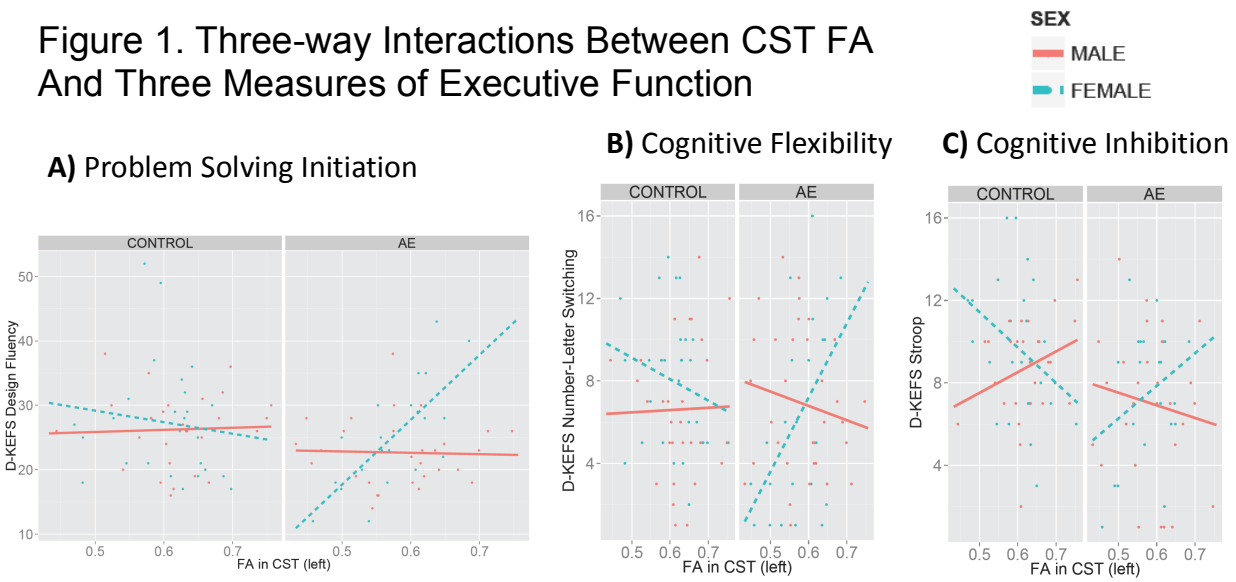
Brain Image Acquisition and Neurobehavioral Assessments:
USC/CHLA: We currently have two CIFASD psychometricians, Max Orozco and Alexy Andrade. Both have approval for neurobehavioral testing and have been evaluating CIFASD participants over the last year. To date, we have evaluated 62 (32 AE, 30 CON) participants at CHLA for the neuroimaging protocol. Of these, 5 have returned for the 2 year follow up appointments.
SDSU: A total of 45 CIFASD participants have been evaluated with neuroimaging and neurocognitive measures, and all neuroimaging data have been transferred to USC/CHLA.
Emory: A total of 48 CIFASD participants have been evaluated with neuroimaging and neurocognitive measures, and all neuroimaging data have been transferred to USC/CHLA.
UMN: A total of 64 CIFASD participants have been evaluated with neuroimaging and neurocognitive measures, and all neuroimaging data have been transferred to USC/CHLA. Of these, 5 have returned for the 2 year follow up appointments.

Facial Imaging and Genetic (saliva) Data Collection at CHLA: We hosted Dr. Ken Jones and Leah Wetherill at our site in December 2014 and examined 29 participants for facial dysmorphology and 3D facial imaging. This brings our cumulative total to 50. We have collected saliva samples for genetic analyses on all CIFASD participants at CHLA/USC.

Brain Image Analyses: Studies and Results

USC/CHLA:
Associations between structural connectivity and cognition in youth prenatally exposed to alcohol: Following prenatal alcohol exposure (PAE), a highly complex pattern of impairments in cognition, self-regulation, adaptive functioning, and associated brain alterations are observed; all of which are included under the term fetal alcohol spectrum disorders (FASD). However, little is known about how white matter connectivity *among* regions is altered by PAE. Diffusion tensor imaging (DTI) MRI assesses white matter tract integrity by measuring restriction of water

Figure 1. Three-way Interactions Between CST FA And Three Measures of Executive Function



Group Differences:
Males: p=0.59
Females: p=0.009*
Sex Differences:
Con: p=0.43
PAE: p=0.011*

Group Differences:
Males: p=0.59
Females: p=0.009*
Sex Differences:
Con: p=0.43
PAE: p=0.011*

Group Differences:
Males: p=0.16
Females: p=0.069#
Sex Differences:
Con: p=0.021*
PAE: p=0.37

diffusion, known as fractional anisotropy (FA). During development, FA increases, likely reflecting the increases in myelination that occur with age. Animal studies have demonstrated sex differences in both the effects of PAE on neurobiology[1,2], as well as myelination development in Controls[3]. However, little is known about how associations between FA and cognition differ between FASD boys and girls. Preliminary results, utilizing along-tract-statistics for 9 white matter tracts, revealed reductions in FA in PAE compared to Control adolescents in the midline of the left cingulum bundle in cingulate gyrus (CGC) and superior portion of the left cerebral spinal tract (CST)[4]. The present study examined associations between FA from these specific points along the CGC and CST, with executive functioning (EF). **Methods:** DTI images (30 directions) were obtained from Control and PAE adolescents (n=121, $\bar{X}$=14.2±2 yrs, 44%F) as part of the Collaborative Initiative on FASD from Los Angeles and Cape Town, South Africa. Three DKEFS measures of EF were examined: Problem-solving/Initiation (Design Fluency), Cognitive Inhibition (Stroop) and Mental Flexibility (Trail Making Test B). Linear mixed-effect modeling was used to examine the effects of Sex, Group (PAE vs. Con), FA (CGC or CST), Age, and their interactions on EF. **Results:** Significant three-way [FA*Group*Sex] interactions were observed (controlling for Age), on all three EF measures with the CST (p's<0.033; **Figure 1)**, but not the CGC. *Post-hoc* analyses revealed that CST associations with EF were altered in PAE compared to Con girls for Design Fluency and Trails (p's<0.01) and a trend for the Stroop (p=0.069). Specifically, PAE girls exhibited a positive correlation between CST FA and EF, whereas Con girls exhibited either a negative or no correlation. In boys, there were no significant differences between Groups for the CST FA association with EF. Additionally, there were opposing sex differences between Groups: 'FA-Stroop' associations were sexually dimorphic for Con (p=.02) but not PAE; and 'FA-Design Fluency' and 'FA-Trails' were sexually dimorphic in PAE (p's<0.012) but not Con. **Discussion:** These novel findings suggest that PAE results in sexually dimorphic alterations in associations between EF and FA. A common pattern was observed across multiple measures of EF, with only PAE girls showing a positive correlation between CST FA levels and EF performance. Previous studies with Controls found a

similar positive correlation between FA levels and EF during early, but not late adolescence in the left anterior corona radiata[5]. Thus, it is possible that the FA-EF association in PAE girls is more indicative of an immature or altered relationship. That is, it is possible that PAE results in reduced specificity of neurocircuitries[6], resulting in positive brain-cognition associations between the motor system (CST) and EF in PAE when these relationships do not typically exist in Con adolescents. The present findings also support region specific results, as brain-EF associations were altered in the CST, but not CGC, in PAE girls. Future analyses are warranted using additional cognitive and white matter measurements to fully elucidate sex-specific effects of PAE, which could enhance specialized interventions. This work was presented at the Organization for Human Brain Mapping conference in Hamburg, Germany, June 2014 (Uban et al.)

Prenatal alcohol exposure and relationships between brain volume, income, and maternal education: In another study conducted with CIFASD II data, we evaluated 87 participants (42 PAE/43 control; age: 8.2-17.7 years) twice, ~2 years apart in the Western Province of Cape Town, South Africa. These children were initially recruited as an epidemiological study of the incidence of FASD among a cohort comprising all children in the first grade at 13 schools, and quantity, frequency and timing (QFT) data were collected retrospectively using time-line follow back procedures at the time of neuroimaging. We evaluated change in white matter and subcortical volumes among these participants. Change in white matter and subcortical volumes were associated with average drinks consumed per week (13.3, 11.1, and 9.1 for 1st, 2nd and 3rd trimesters respectively), and the regional pattern varied by trimester of exposure. Importantly, we also show significant relationships between maternal education and family income and change in white matter and subcortical volumes **(Fig. 1-2)**. Interestingly, these findings overlap with findings from a study we conducted in a large US sample of 1,099 typically developing children 3-20 years old. In this large sample, family income was highly significantly associated with brain surface area**,** and this effect was observed in some of the same brain regions where associations between change in white matter were associated with family income in the South Africa sample. Overall, these preliminary parallel findings suggest common effects of SES on brain development across geographical regions and cultural environments. This work will be presented at the Organization for Human Brain Mapping conference in Honolulu, June, 2015 (Gautam et al.).

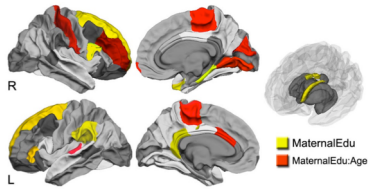


Figure 1: Significant change in regional white matter and subcortical volumes correlated with Maternal education. Regions showing significant interactions with Age and Maternal education are also shown.

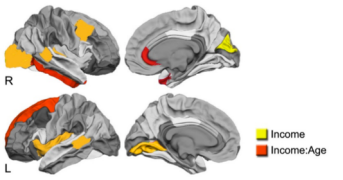


Figure 2: Regional white matter volume changes that are significantly correlated with family income (yellow). Significant interactions between income and age are also shown (red).

Emory: Using the data collected at the Atlanta site, Dr. Coles and other investigators carried out a preliminary analysis of functional connectivity. Resting-state (eye closed) fMRI data were acquired from 15 control (8M7F, Age1st=13.1±2.9) and 15 PAE (9M6F, Age1st=12.7±3.7) adolescents, CIFASD III participants studied at their site. As shown in Fig.3, significant reduction of functional connectivity was observed in 6 out of the 7 networks (except salient network) in the exposed group, demonstrating a long-term and large-scale compromise of network capacity associated with PAE. Together with the reduced functional connectivity in several attention regions of the brain, these results could provide some informantion about the neural underpinnings of cognitive and attention deficits regularly seen in PAE population, and support the general hypothesis of PAE associated large-scale network-disconnectivity. This

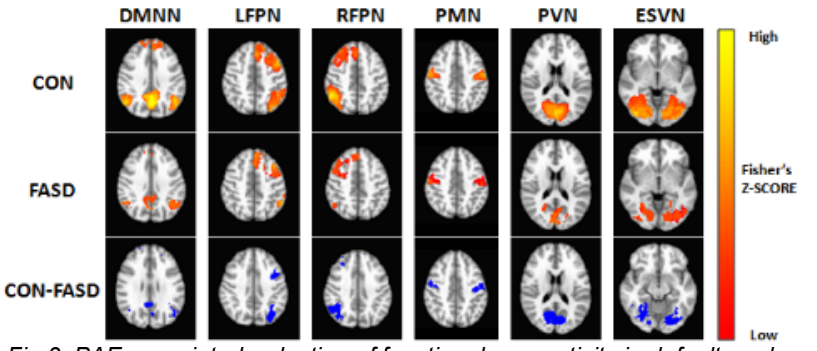


Fig 3. PAE associated reduction of functional connectivity in default mode (DMN), left prefrontal/parietal (LPFN), right prefrontal/parietal (RPFN), primary motor (PMN), primary visual (PVN), and extra striate visual (ESVN) networks. Blue regions are voxels with a significant connectivity reduction in the PAE group.

work was accepted by the International Society for Magnetic Resonance in Medicine Annual Meeting in Toronto, Canada, May 30-31, 2015.

UMN: Dr. Wozniak and colleagues have been working on two imaging projects since the completion of the first phase of imaging data collection. First, they are continuing to refine their methods for characterizing whole-brain resting-state functional connectivity in children with prenatal alcohol exposure (PAE). They have previously applied these methods in Wozniak et al. 2013. The whole-brain methods rely on the 6-minute resting-state (non-task) fMRI data being collected at all four human sites. The analyses focus on characterizing global aspects of network efficiency in PAE based on the understanding that alcohol has widespread effects on the developing brain and based on previous diffusion tensor imaging (DTI) data showing widespread disturbances in microstructural connectivity. Global metrics of each individual's network status / efficiency are being used in characterizing the severity of effects of PAE and in understanding the underlying neurodevelopmental correlates of abnormal cognitive functioning in PAE. We have provided the data from all sites and Dr. Wozniak's group is proceeding with resting-state data integration from 164 participants. Challenges include modifying the data processing pipeline to function with data from four different MRI scanners and integrating the results. They have made substantial progress in this and will be able to run the first sets of analyses within the next several months. We have also provided the structural MRI data from all four clinical sites as well as the structural MRI data from phase II to Dr. Wozniak's group, and they have been working to combine these data for a very large-scale analysis of cortical thickness (expected to include 400+ individuals). This set of analyses will have ample power to address a key unresolved question in PAE at the moment: whether cortical thickness is altered differentially at different points in development in PAE.

Summary of Findings: Our studies over the last year have yielded some important insights into the impact of prenatal alcohol exposure and how it impacts brain and cognitive development. Some of the studies in progress are described above, and, our published works are listed below. We have shown that trajectories of brain change are different in youth with prenatal alcohol exposure with respect to brain structure, and brain function. We have shown that functional and structural brain connectivity are altered in FASD. Together, these findings are promising for potential impact of early intervention, given that the brain in all individuals (whether or not exposed prenatally) is "wired" throughout development, and that experiences within the environment help shape brain connectivity. Notably, factors independent from prenatal exposure (i.e., SES) are also related to brain change, further emphasizing potential impact for interventions targeting deficiencies in the developmental environment could impact change over time.

3. For this reporting period (April 1, 2014 - March 1, 2015), is there one or more **Revision/Supplement** associated with this award for which reporting is required? N/A

4. What **opportunities for training and professional development** has the project provided?

Dr. Kristina Uban, a postdoctoral fellow in the Developmental Cognitive Neuroimaging Laboratory at USC/CHLA obtained an NRSA fellowship from NIAAA (F32 AA022561) to study pubertal hormones and sex differences in the CIFASD population. Her research project will assess hormone function in PAE boys and girls, and hormones will be integrated with measures of structural and functional brain connectivity. Integrated brain and hormone measures will be correlated with measures of cognitive performance (i.e., WISC-IV).

5. How have **results** been **disseminated** to communities of interest?

a. We published a commentary in Nature late last year focused on informing the general public about the safety of drinking during pregnancy (Sowell ER, Charness ME, Riley EP, Pregnancy: No safe level of alcohol. *Nature*. 2014 Sep 11;513(7517):172).

b. We have also been invited to prepare a Science and Society article for *Trends in Cognitive Sciences*, focused on summarizing the scientific evidence for an impact of low-to-moderate intake on cognitive and brain development. Science and Society articles are short pieces (up to 1500 words) that are intended to highlight scientific topics of greater societal relevance. They are aimed at a broad audience and frequently stimulate debate and attract media coverage.

6. What do you **plan to do for the next reporting period** (March 1, 2015 - April 1, 2016) to accomplish the goals?

In the next reporting period, we plan to continue recruiting participants and administer neurobehavioral assessments until we meet the project goals for the 2nd time point of neuroimaging. We will continue to flesh out analyses already begun (described above), and develop new analysis strategies that will combine face, brain, and neurocognition (see preliminary study described below).

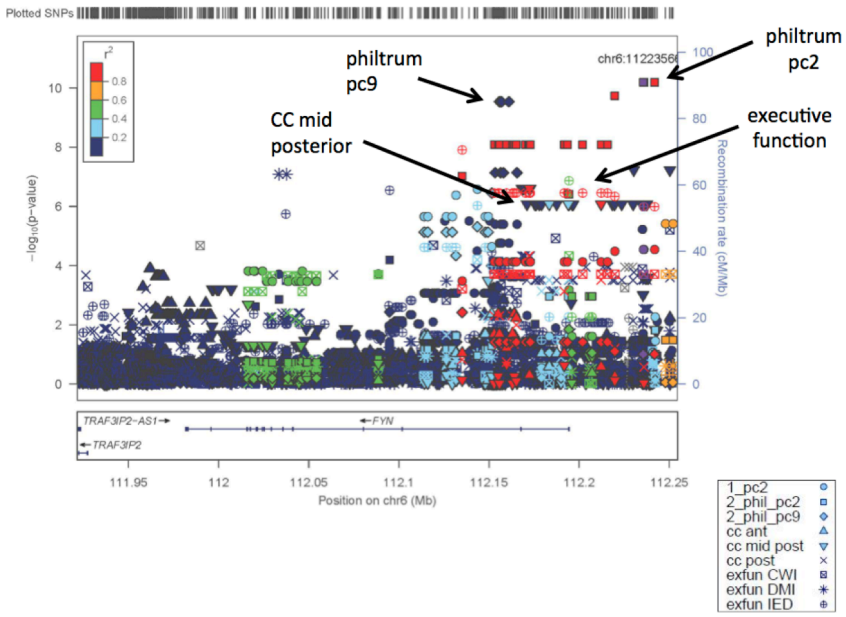


Figure 4: Results from preliminary analyses showing the predictors of genotype from a priori selected phenotypes. Figure created by T. Foroud and L. Wetherill

7. Describe your project's **interrelation** with aims of the CIFASD consortium its other projects.

This project is closely integrated with the A. Neuroimaging; B. Facial Dysmorphology; and C. Genetics projects. Participants are shared among these projects and the projects are set up to allow for extensive cross-discipline / cross-project data integration.

We recently participated in a "work-group" including the Neuobehavior, 3D facial imaging, neuroimaging, and genetics projects to integrate neuroimaging findings with those from basic science. This group has identified candidate genes from zebrafish and human cell models as being associated with susceptibility to the harmful effects of prenatal alcohol exposure. As a group, we determined the most meaningful variables from the various project domains (a priori hypotheses). The preliminary dataset consisted of 10 principal components describing facial shapes, 10 principal components describing the philtrum, 3 executive function tasks and 5 corpus callosum brain volumes. Genetic analyses were conducted to examine if there was a genotype by alcohol exposure interaction; i.e., is the genotype, in the presence of prenatal alcohol exposure, protective of the phenotype (e.g., less dymorphic face, better executive function, increased brain volume). Preliminary results for *FYN*, a member of the Src kinase family, demonstrated moderately significant association of genetic markers in the gene with facial shapes, executive function, and brain volume, in the presence of prenatal alcohol exposure. These findings formed the basis of our upcoming Symposium to be presented at RSA in June 2015.

8. **Publications [Accepted & In Press]**

- Gautam P, Nuñez SC, Narr KL, Mattson SN, May PA, Adnams CM, Riley EP, Jones KL, Kan EC, **Sowell ER.** Developmental Trajectories for Visuo-Spatial Attention are Altered by Prenatal Alcohol Exposure: A Longitudinal FMRI Study. *Cereb Cortex.* 2014 Aug 4. [Epub ahead of print]
- Nguyen TT, Glass L, Coles CD, Kable JA, May PA, Kalberg WO, **Sowell ER**, Jones KL, Riley EP, Mattson SN; CIFASD. The clinical utility and specificity of parent report of executive function among children with prenatal alcohol exposure. *J Int Neuropsychol Soc*. 2014 Aug;20(7):704-16
- 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. *Neuroimage Clin.* 2014 Jun 4;5:19-27
- Ware AL, Glass L, Crocker N, Deweese BN, Coles CD, Kable JA, May PA, Kalberg WO, **Sowell ER**, Jones KL, Riley EP, Mattson SN; CIFASD. Effects of prenatal alcohol exposure and attention-deficit/hyperactivity disorder on adaptive functioning. *Alcohol Clin Exp Res*. 2014 May;38(5):1439-47
- **Sowell ER**, Charness ME, Riley RP. Pregnancy: No safe level of alcohol. *Nature.* 2014 Sep 11; 513(7517):172

9. **Publications [In Preparation & Submitted]**

- Joshi SH, Narr KL, Kan E, Woods RP, Toga AW, Mattson SN, Riley EP, Jones KL, Adnams CM, May PA, O'Connor MJ, and **Sowell ER**. Reduced Gyrification in Fetal Alcohol Syndrome. In Prep.
- Uban KA, Herting MM, Kan EC, Colby JB, Gautam P, Mattson SN, Riley EP, **Sowell ER**. Alterations in structural connectivity in adolescents with a fetal alcohol spectrum disorder are sexually dimorphic. In Prep.

10. **Poster Abstracts and Presentations**

- Uban KA, Namer L, Gautam P, Herting MM, Colby JB, Kan EC, Adnams CM, May PA, Narr KL and **Sowell ER**. Hemispheric Asymmetry in White Matter Microstructure in Adolescents with Fetal Alcohol Spectrum Disorder (FASD). Presented at the 2nd Annual Flux Congress Meeting, Universal City, CA, Sept 2014
- 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. Presented at the 3rd annual Research Society on Alcoholism Scientific Meeting, Bellevue, WA, June 2014
- Uban KU, Lynch KL, Andrade AF, Herting MM, Gautam P, Nunez SC, Colby JB, Kan EC, Adnams CM, May PA, Narr KL, Mattson SN, Riley EP, **Sowell ER**. Associations between structural connectivity and cognition youth prenatally exposed to alcohol. Presented at the 20th Annual Meeting of the Organization for Human Brain Mapping, Hamburg, Germany, June 2014.
- Gautam P, Warner TD, Kan EC, **Sowell ER**. Executive function and cortical thickness in youth prenatally exposed to cocaine, alcohol & tobacco. Presented at the 20th Annual Meeting of the Organization for Human Brain Mapping, Hamburg, Germany, June 2014.
- Gautam P, Lebel C, Narr KL, Mattson SN, May PA, Adnams CM, Riley EP, Jones KL, Kan EC, **Sowell ER**. Sub cortical volume changes and brain-behavior relationships in youth prenatally exposed to alcohol. Presented at the 20Th Annual Meeting of the Organization of Human Brain Mapping, Hamburg, Germany, June 2014
- Ji, B, Li, Z, Coles, CD, Kable, JA, Zhang, R, & Hu, "Reduction of functional connectivity in adolescents prenatally exposed to alcohol" to be presented at International Society for Magnetic Resonance in Medicine Annual Meeting, Toronto, Canada, June, 2015.
- Brain Imaging in FASD: From Animal Studies to Human Findings. To be presented by Kristina Uban, PhD at the 6th International Conference in FASD, Vancouver, BC, Canada, March 6, 2015.

Principal Investigator(s): Sarah N. Mattson
Institution(s): San Diego State University
CIFASD Project Title: A Multisite Neurobehavioral Assessment of Fetal Alcohol Spectrum Disorders (FASD)
Grant Number: 5 U01 AA014834-11

1. What are the **major goals** of the project?

Aim 1. Use existing data to develop a tiered or hierarchical approach to identification of affected cases. This aim will determine the measures, from all four clinical domains of CIFASD (neurobehavior, dysmorphology, 3D facial imaging, and brain imaging) that could be used clinically to identify alcohol-affected children.

Aim 2. Test the specificity and sensitivity of the model developed in Aim 1 in children ages 10-16. A battery of standardized neurobehavioral tests will be administered to subjects in three subject groups (alcohol-exposed, AE; non-exposed Controls; and non-exposed clinically-referred Contrast subjects) at four sites. Sensitivity (AE vs. Control) and specificity (AE vs. Contrast) will both be tested. Data will be combined with data from other CIFASD projects.

Aim 3. Test the utility of the model in younger children, ages 5-7. A similar battery of age-appropriate standardized neuropsychological tests will be administered to young children in the same three subject groups at three of the four sites. Sensitivity and specificity will be tested as in Aim 2.

Aim 4. Targeted assessment of memory function. In Phase I and II, our test batteries focused heavily on executive function, which proved to be an important domain in our preliminary neurobehavioral profile. Past studies and some preliminary data suggest that memory is another important domain and further study, including tests of both sensitivity and specificity, is warranted.

2. What was **accomplished** under these goals?

All sites are actively collecting data and are making progress toward our overall subject recruitment goals. See **Table 1**.

Neurobehavioral Progress 2012-2017

Site	Goal Per Group	Alcohol-Exposed	Control	Contrast
SDSU Old	44	33	42	31
SDSU Young	44	12	23	18
UMN Old	44	40	38	26
UMN Young	44	25	30	20
Emory Old	44	23	29	29
Emory Young	44	25	20	17
USC Old	44	17	19	n/a
	All Groups & Ages	Alcohol-Exposed	Control	Contrast
Tested Total	517	175	201	141
Goal Total	880	308	308	264

We are making progress on all stated specific aims. **Aim 1**, which is to develop a hierarchical approach to identification of affected children was initially difficult to carry out. However, in the last year, we took a different analysis strategy and have made considerable progress. For this aim, we our goal was to improve identification by developing a brief and clinically relevant screening tool that is easily deployed in clinical settings. To this end, we examined data from CIFASD, phase II, which comprised >1200 neurobehavioral and dysmorphology variables from >400 subjects, including alcohol-exposed children, typical controls, and children with other clinical concerns (ADHD or IQ<88). We created a decision tree model that maximized both sensitivity and specificity and minimized the number of measures required. Data reduction was conducted by selecting variables and cutoff criteria that maximally separated groups (alcohol-exposed from non-exposed; alcohol-exposed from non-exposed with other behavioral problems) and resulted in the most accurate predictive values (highest number of accurately identified subjects). Variables were added until sample sizes prohibited further discrimination. We sought a model that

accurately classified >80% of subjects and capitalized on the interaction between the dimensions of neurobehavioral and dysmorphology data. The resulting model involves only 1 child measure (IQ), 2 parent questionnaires (CBCL, VABS) and a dysmorphology exam. Thus it is clinical useful and very efficient. We calculated overall accuracy, sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for 2 entry points. The first entry point (via psychologist or other mental health professional) of the decision tree obtained an 81.1% overall accuracy (sensitivity: 69.3%, specificity: 87.9%; PPV: 83.0%, NPV: 80.3%), see **Figure 1**. The second entry point (via a dysmorphologist) of the decision tree obtained an 80.6% overall classification accuracy (sensitivity: 75.8%, specificity: 84.0%; PPV: 76.4%, NPV: 83.5%), see **Figure 2**. Classification accuracies at both entry points of the model were significantly higher than chance (*p*s < .001).

Figure 1. Hierarchical decision tree (aim 1) developed using CIFASD II data, entry point 1 (clinical psychologist or other mental health professional).

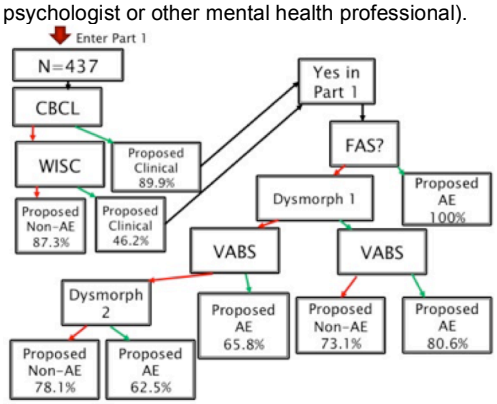
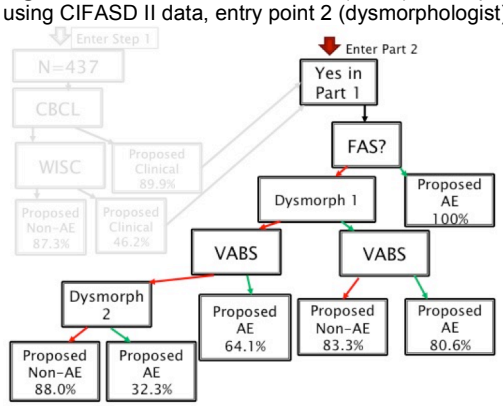


Figure 2. Hierarchical decision tree (aim 1) developed using CIFASD II data, entry point 2 (dysmorphologist).



To validate the model, and address **Aims 2 & 3**, we applied it to a different dataset of neurobehavioral and dysmorphology data collected from two different age groups from CIFASD Phase III, which is ongoing. Subjects were 454 children aged 5-7y (C; *M*=6.6) and adolescents aged 10-16y (A; *M*=13.4), and included alcohol-exposed children, typical controls, and children with other clinical concerns (e.g., ADHD, learning or behavior disorders). In the older age group and the 1st entry point, the model correctly classified 76.9% (sensitivity: 68.3%, specificity: 81.6%; PPV: 87.5%, NPV: 72.9%). At the 2nd entry point, 83.0% of the sample was correctly classified (sensitivity: 70.0%, non-AE: 91.7%; PPV: 84.9%, NPV: 82.1%). All values were found to be significantly higher than chance (*p*s < .001). In addition, each step of the decision tree was found to be statistically significant (*p*s < .05).

In the younger age group at the 1st entry point, the model correctly classified 76.7% of the sample (AE: 70.7%, non-AE: 80.6%; PPV: 87.9%, NPV: 71.4%). At the 2nd entry point, 82.1% of the sample was correctly classified (AE: 63.8%, non-AE: 93.4%; PPV: 85.7%, NPV: 80.7%). When entering at the 1st entry point, z-test analyses showed that all values were significantly higher than chance (*p*s < .001). When entering at the 2nd entry point, all values except sensitivity (*p* = .057) were found to be significantly higher than chance (*p*s < .001). Each step of the decision tree was found to be statistically significant (*p*s < .05) except at the node employing a measure of general cognitive ability (*p* = .143) within the 1st entry point.

The step-wise decision tree model is superior to a simpler simultaneous analysis of the same data, which we have done before, because differences between subgroups of subjects can be exploited. This approach maximizes the power of our large multi-dimensional dataset with the ultimate goal of simplifying clinical diagnosis. This project (both model development and model validation) will be presented at the 2015 RSA meeting in June.

In keeping with Aim 3, we have also examined CIFASD III data for differences based on age. CIFASD III includes 2 age groups, as described above. In addition, we also included sex as a

factor in these analyses. Data regarding neuropsychological function, psychopathology, and behavior were analyzed using a 2 (group: AE vs. CON) x 2 (age: young vs. old) MANCOVA. Overall N=326. Results indicated a significant omnibus effect of exposure for all analyses. Follow-up tests revealed significant main effects of exposure (AE<CON) on all variables. A significant main effect of age was found in the neuropsychological performance and adaptive functioning analyses, but not for psychopathology. Follow-up tests revealed that older children had significantly poorer scores than younger children on two language tasks, and one measure of visuospatial ability. Older children also had significantly poorer communication and socialization scores than younger children. There were no significant main effects of sex for any analysis, and there were no significant interactions in any of the omnibus analyses. Thus, these results replicate previous reports of impaired neuropsychological and behavioral functioning in alcohol-exposed children but do not support differential effects of age and sex on this population. Clinically, the lack of interactions in these findings suggests consistent performance patterns in alcohol-exposed children and adolescents, regardless of age and sex. Further, these cross-sectional data support the use of the same across a broad range of alcohol-exposed subjects, which helps as we design our improved diagnostic batteries (see above). These data were presented at the 2014 RSA meeting and a complete draft of the paper has been completed.

3. For this reporting period (April 1, 2014 - March 1, 2015), is there one or more **Revision/Supplement** associated with this award for which reporting is required?

Nothing to Report.

4. What **opportunities for training and professional development** has the project provided?

Nothing to Report.

5. How have **results** been **disseminated** to communities of interest?

Nothing to Report.

6. What do you **plan to do for the next reporting period** (March 1, 2015 - April 1, 2016) to accomplish the goals?

During the next funding year we plan to continue data collection at all 4 sites, and analyze new and existing data to meet our aims. We are preparing 5 manuscripts, as listed below. Our primary focus is the manuscript describing the hierarchical decision tree.

7. Describe your project's **interrelation** with aims of the CIFASD consortium its other projects.

This project is strongly integrated with the rest of CIFASD in several ways. On the site level, four sites are participating in this component and all are administering neurobehavioral testing (4 have older cohorts; 3 of these have younger cohorts). On the project level, subjects tested for Phase III are assessed as part of the 3D facial imaging (T. Foroud) and a subset are included in the brain imaging study (E. Sowell) projects as well as the genetics developmental project (T. Foroud) and data from all projects will be analyzed. Finally, on the level of the cores, this project integrates with the Dysmorphology core (K. Jones), the Informatics core (W. Barnett), and the Administrative core (E. Riley). All children receive a standardized dysmorphology examination, which will provide diagnostic information, aid subject classification, and provide data for the aims of the Dysmorphology core. Finally, our specific aims will be supported by the Administrative core by utilizing the resources provided by the core such as statistical support and teleconferencing.

The projects involved in CIFASD are also interrelated in terms of data analysis. Neurobehavioral data have been integrated into several analyses directed by other projects. In particular, neurobehavioral data are included in recent studies from the facial imaging group and the brain

imaging group. In keeping with our first specific aim, the neurobehavioral project, together, with the dysmorphology core is developing a decision tree model, as described above. Further we are preparing a paper (in early stages, listed below) combining neurobehavioral and brain data to address the effects of prenatal alcohol exposure on memory function (Aim 4).

8. **Publications** **[Accepted & In Press]**

Publications Reported for this Reporting Period

NIH Public Access Compliance	Citation
PMC Journal In Process	Gautam P, Nuñez SC, Narr KL, Mattson SN, May PA, et al. Developmental Trajectories for Visuo-Spatial Attention are Altered by Prenatal Alcohol Exposure: A Longitudinal FMRI Study. Cereb Cortex. 2014 Aug 4;PubMed PMID: 25092900. [Epub ahead of print]
Complete	Nguyen TT, Glass L, Coles CD, Kable JA, May PA, et al. The clinical utility and specificity of parent report of executive function among children with prenatal alcohol exposure. J Int Neuropsychol Soc. 2014 Aug;20(7):704-16. PubMed PMID: 25033032; NIHMSID: NIHMS620944; PubMed Central PMCID: PMC4228981.
Complete	Ware AL, Glass L, Crocker N, Deweese BN, Coles CD, et al. Effects of prenatal alcohol exposure and attention-deficit/hyperactivity disorder on adaptive functioning. Alcohol Clin Exp Res. 2014 May;38(5):1439-47. PubMed PMID: 24655090; NIHMSID: NIHMS567036; PubMed Central PMCID: PMC3999227.
Complete	Cardenas VA, Price M, Infante MA, Moore EM, Mattson SN, et al. Automated cerebellar segmentation: Validation and application to detect smaller volumes in children prenatally exposed to alcohol. Neuroimage Clin. 2014;4:295-301. PubMed PMID: 25061566; PubMed Central PMCID: PMC4107371.

9. **Publications** **[In Preparation & Submitted]**

Mattson, Goh, et al., and the CIFASD. Relation Between Age and Internalizing and Externalizing Behaviors in Children with Prenatal Alcohol Exposure. Manuscript in preparation (data being analyzed), CIFASD concept proposal form submitted in January 2015.

Mattson, Jones, Goh, Doyle, et al., and the CIFASD. Developing a decision tree for clinical identification of children affected by prenatal alcohol exposure. Manuscript in preparation (paper being written), CIFASD concept proposal form submitted in January 2015.

Mattson et al., and the CIFASD. Neuropsychological and behavioral functioning do not vary by age & sex in FASD (Aim 3). Manuscript in preparation (paper being written), CIFASD concept proposal form submitted in January 2015.

Mattson, Panczakiewicz, Gross, et al., and the CIFASD. Targeted assessment of memory function in FASD (Aim 4). Manuscript in preparation (data being analyzed), CIFASD concept proposal form submitted in January 2015.

Mattson, et al., and the CIFASD. Brain-behavior relations in FASD: Focusing on memory function. Manuscript in preparation (data base under construction), CIFASD concept proposal form submitted in January 2015.

10. **Poster Abstracts and Presentations**

Glass, L., Panczakiewicz, A., and Mattson, S.N. (2014). Effects of age and sex on behavioral and neuropsychological functioning in FASD. Presented at the Research Society on Alcoholism meeting, Bellevue, June 2014. Alcoholism: Clinical and Experimental Research, 38, Supplement S1, 178A. doi: 10.1111/acer.12451, Published online 30 May 2014. http://onlinelibrary.wiley.com/doi/10.1111/acer.12451/abstract

Adnams, C.M., Pomario, T., Corbett, C., Santilli, L., May, P.A., Riley, E.P., and Mattson, S.N. (2014). Behavior in secondary school learners with prenatal alcohol exposure in South Africa. Presented at the Research Society on Alcoholism meeting, Bellevue, June 2014. Alcoholism: Clinical and Experimental Research, 38, Supplement S1, 177A. doi: 10.1111/acer.12451, Published online 30 May 2014. http://onlinelibrary.wiley.com/doi/10.1111/acer.12451/abstract

Suttie, M., Wetherill, L., Jones, K., Coles, C., Sowell, E., Mattson, S., Riley, E., Foroud, T., and Hammond, P. (2014). Analysing genetic and teratogenic effects of alcohol on facial form. Presented at the Research Society on Alcoholism meeting, Bellevue, June 2014. Alcoholism: Clinical and Experimental Research, 38, Supplement S1, 357A. doi: 10.1111/acer.12452, Published online 30 May 2014. http://onlinelibrary.wiley.com/doi/10.1111/acer.12452/abstract

Wetherill, L., Hammond, P., Suttie, M., Jones, K., Coles, C., Sowell, E., Mattson, S., Riley, E., Eberhart, J., Dou, X., Charness, M.E., and Foroud, T. (2014). Gene x prenatal alcohol exposure: preliminary results in SRC family kinase and PDGF pathways. Presented at the Research Society on Alcoholism meeting, Bellevue, June 2014. Alcoholism: Clinical and Experimental Research, 38, Supplement S1, 358A. doi: 10.1111/acer.12452, Published online 30 May 2014. http://onlinelibrary.wiley.com/doi/10.1111/acer.12452/abstract

Principal Investigator(s): Kathleen K. Sulik
Institution(s): University of North Carolina
CIFASD Project Title: Craniofacial and CNS Pathology in a Mouse FASD Model
Grant Number: 5 U01 AA021651-03

1. What are the **major goals** of the project?

The primary objective of this work is to make clinically significant discoveries regarding prenatal alcohol (ethanol) exposure-induced pathology involving the brain and face, with the long-range goal of improving clinical practice and reducing the incidence of alcohol-induced birth defects. **The overall hypothesis that is being tested in a mouse model 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 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. This last Aim has been expanded to include examination of genetic and environmental modifiers of alcohol teratogenicity with the objectives of identifying clues regarding alcohol's teratogenic mechanism(s) and better understanding individual variability in teratogenic response.

2. What was **accomplished** under these goals?

During this funding period (6/01/2014-5/31/15) our publications have included 1 review chapter and 4 full papers, one of which is currently in press. A total of 11 abstracts have also been published or submitted during this funding period.

Our additional research progress includes the following:

- Brain/face correlative analyses are being conducted in collaboration with Dr. Hammond and Mike Suttie (see Figure 1). Manual segmentation of MR images; used in the previous GD7/GD8.5 comparison (Lipinski et al, 2012) to extract regional brain components is time consuming and subject to intra and inter-rater inconsistencies. A more reliable and less laborious method is to use an automated segmentation technique. For this we utilize open-source software packages; NiftyReg and NiftySeg developed by Centre for Medical Image Computing (CMIC) at University College London (UCL). NiftyReg contains tools to perform rigid, affine and non-linear registration; a prerequisite for the segmentation process, whilst NiftySeg is capable of image segmentation, bias field correction and cortical thickness estimations. The image segmentation algorithm (Van Leemput et al, 1999) is based on an Expectation-Maximization procedure initialized from multiple atlases. This automated multi-atlas approach hierarchically selects combinations of best-fit atlases to provide accurate and reproducible segmentations.

At current standing we have processed and segmented GD10 (n=69) and GD11.5 (n=31) ethanol exposed mice with 42 GD11.5 images remaining. Minor technical errors and image issues requiring further pre-processing delayed the segmentations. However these have been resolved and the batch processing is ongoing. We expect the remaining images to be completed by mid to late March if no further issues arise.

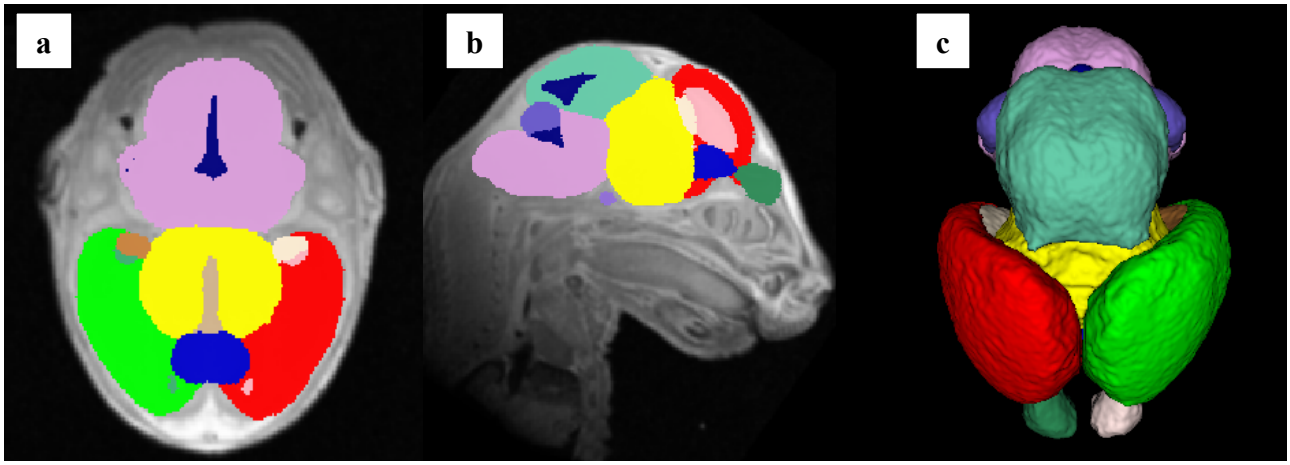


Figure 1 Example of the extracted regional brain components of a GD10 exposed mouse imaged at GD17 and segmented using automated NiftySeg and NiftyReg software. (a) Axial view (b) mid-sagittal view (c) 3D rendering of the segmented components.

- Following up on studies showing that in mice, insult to the Shh pathway underlies alcohol-induced teratogenesis on GD 7, we have conducted a similar GD 9.25 study. Because the role of Shh signaling in limb development is well understood and the limb is vulnerable to alcohol-induced defects, this study has focused on limb dysmorphology. As shown in Figure 2, the severity and frequency of postaxial reduction defects are much greater in mice that are Shh haploinsufficient than in normozygous animals. These findings have provided the foundation for a U54 subproject proposal that is a collaborative effort between our laboratory and that of Kevin Williams at NCCU. In this grant application we propose to conduct RNA seq analyses to identify genes that are both upstream and downstream of Shh whose modification may also influence alcohol's teratogenicity.

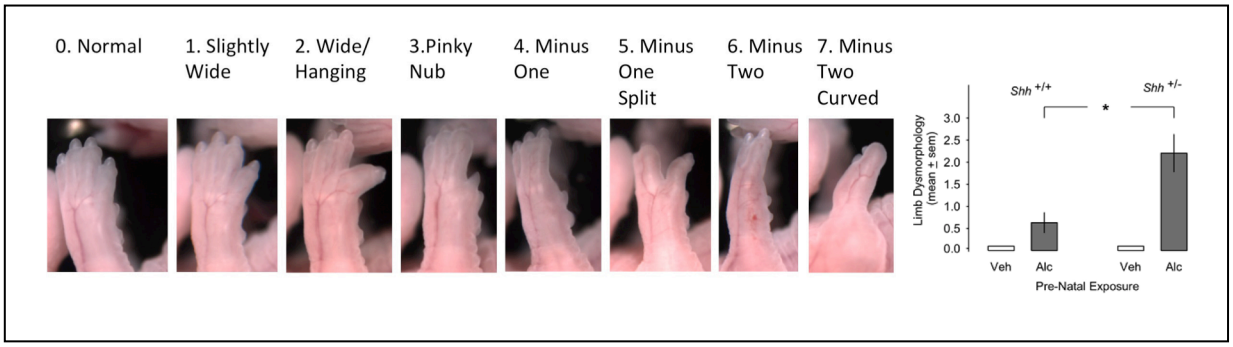


Figure 2. Shown are the right forelimbs of perinatal mice from Shh normozygous/heterozygous matings. Dysmorphology scores were assigned based on a scale that rated postaxial deficiency (0=normal to 7=most severe). Animals that were haploinsufficient for Shh were more sensitive to alcohol's teratogenesis than wild types as evidenced by the frequency and severity of their forelimb defects.

- As described in our face-to-face 2014 CIFASD meeting, we are conducting a study directed cannabinoids. This study rests on the facts that new "designer" cannabinoids are extremely potent, little teratogenicity data exists, and the potential for co-exposure to alcohol is very high. Our initial focus has been on administration of the synthetic cannabinoid, CP-55,940, on GD 8 in mice, and establishment of a dose-response curve (Figure 3).

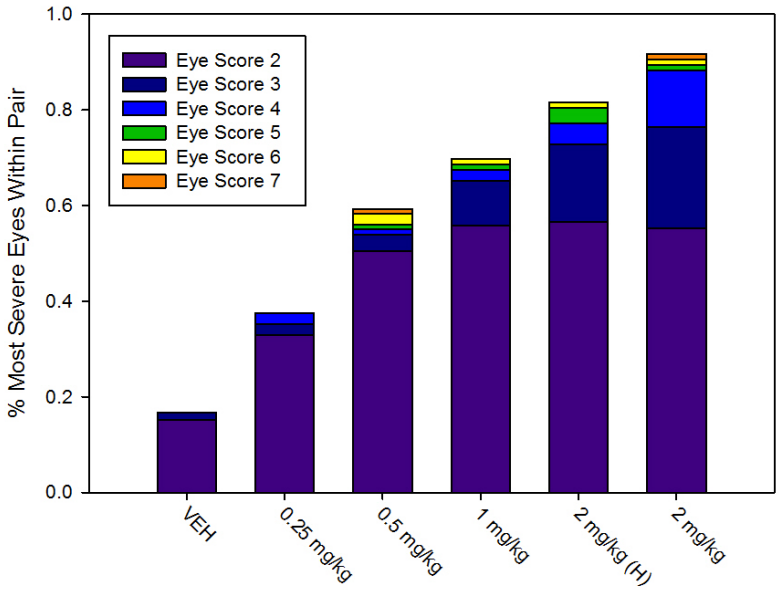


Figure 3. CP 55, 940 dose response analyses illustrate teratogenicity at maternal dosages ranging from 0.125 to 2.0 mg/kg. Scoring for defects is based on the severity of ocular defects. 15% of control C57Bl/6J mice present with a spontaneous eye defect, most of which are subtle. CP 55,940 induced a significantly greater number and severity of eye defects in a dose-dependent manner.

As shown in Figure 4, single maternal dosages of 0.5 or 1.0mg.kg induce CNS and craniofacial malformations. This work has provided the foundation for submission of a grant proposal in response to a NIDA RFA. This proposal includes teratogenesis studies of additional synthetic cannabinoids.

Importantly, preliminary alcohol/CP 55,940 co-exposure studies suggest that, indeed, there is synergy between these drugs relative to induction of birth defects. More specifically, a 0.25mg/kg dosage of CP 55940 alone yielded ocular dysmorphology in 38% of GD 17 fetuses from 11 litters; a 2.8g/kg dosage of alcohol alone in 6 litters yielded 45% ocular dysmorphology; and the combination in 6 litters caused ocular dysmorphology in 98% of the fetuses. With ocular dysmorphology being a readily assessed endpoint that is indicative of CNS damage, and with the damage occurring at a time that precedes typical pregnancy recognition these preliminary findings are highly significant.

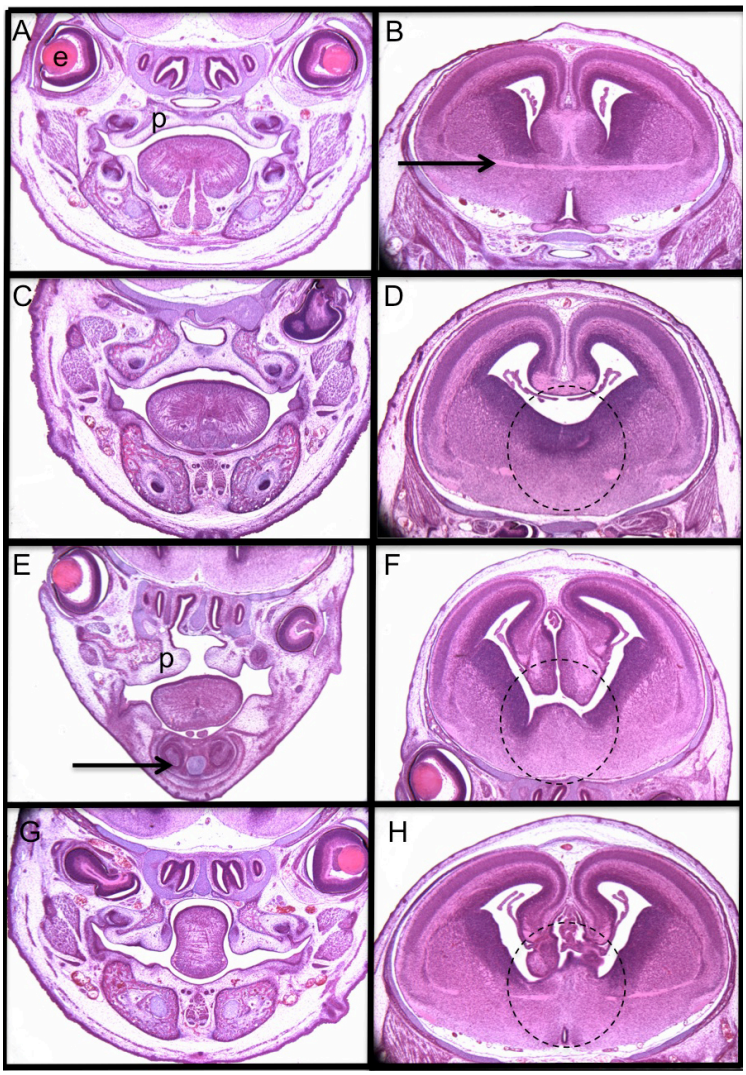


Figure 4. Histological sections of Control (A,B), and CP 55,940-treated GD 17 fetal mice illustrate that maternal dosages of 0.5 mg.kg (C-F) and 1.0 mg/kg (G,H) administered on GD 8 yield abnormalities of the brain, eyes and palate, and mandible. Figures in the left column are through the center of the eyes (e), while those in the left column are through the brain at the level of the anterior commissure (arrow in B). Notable are varying degrees of ocular abnormality involving the right and/or left eyes of the treated animals. Additionally, the fetuses shown in E and G each have secondary palatal clefts (compare to the closed palate [p] in the control specimen). This is accompanied by a small mandible in the fetus shown in E. The brain abnormalities in the fetuses shown in D-F are most evident in the ventral midline (dashed circles) and include septal region deficiencies.

3. For this reporting period (April 1, 2014 - March 1, 2015), is there one or more **Revision/Supplement** associated with this award for which reporting is required?

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 addresses the need for a more complete understanding of the genetic and environmental factors that influence alcohol's teratogensis. 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 (6/01/2014-5/31/15) include:

- Conduct of basic research in mice regarding the potential teratogenic interactions between high potency cannabinoids and alcohol, with an emphasis on establishing baseline cannabinoid data (described above)
- Attended the FASDSG and RSA meetings, Bellevue Washington, June 2014

- Participated in the CIFASD biannual meetings
- Presented a poster at the International Cannabinoid Meeting in Baveno Italy, June, 2014; Received Student Poster Award: 3rd Place, Best Poster, Postdoctoral Category
- Presented a poster at the Carolina Cannabinoid Collaborative Conference, Winston Salem NC, Nov 2014
- Helped prepare and revise an R01 application (SE Parnell, PI) entitled "Teratogenicity of Early Gestational Synthetic Cannabinoid Exposure in Mice" in answer to a NIDA RFA
- Will present a UNC Bowles Alcohol Research Center seminar in March, 2015
- Preparing a full paper on CP-55,940 teratogenicity in mice for submission during this funding period
- Invited to participate in a Gordon Research Seminar on Cannabinoid Function in the CNS in 2015; recipient of $600 Carl Storm Fellowship

Discussion: Dr. Gilbert is making substantial progress in the teratology discipline, has made very important discoveries regarding the teratogenic potential of the synthetic cannabinoid, CP-55,940, and has begun rodent co-exposure studies with this drug and alcohol. Specifics regarding her findings are described above in the main portion of this progress report. This line of investigation is novel, in keeping with the goal of increased NIAAA and NIDA interaction, and promises to serve as a solid foundation for Dr. Gilbert's academic research career. Dr. Gilbert continues to demonstrate the initiative, enthusiasm and drive that exemplify a successful scientist.

Interrelation with the Aims of the Consortium and Other Projects: With identification of factors that may interact with ethanol to cause birth defects, these studies are most complementary to those of the Eberhart and Faroud groups.

Plans for the next year:

- Prepare and submit a full paper on CP-55,940/alcohol co-exposure studies
- Prepare a pathway to independence award (K99/R00) application for the June 2015 deadline
- Present at Gordon Conference in Lucca Italy in May, 2015

Publications (Abstracts)

- **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, Baveno, Italy, June 2014.
- **Gilbert MT**, Parnell SE, Fish EW, Baker LK, Sulik KK. A Mouse Model Of Early Prenatal Cannabinoid Exposure Shows Dose-Dependent Synthetic Cannabinoid Teratogenicity, Carolina Collaborative Cannabinoid Conference, Winston Salem, NC, Nov. 2014.
- Parnell SE, Fish EW, **Gilbert MT**, Wieczorek LA, Williams KP, Sulik KK. Murine-based studies of FASD susceptibility genes provide mechanistic data, 6th International Conference on FASD, Vancouver, Canada, March 2015.

4. What **opportunities for training and professional development** has the project provided?

In addition to Dr. Marcoita Gilbert, a postdoctoral fellow who is supported by the Diversity Supplement described above, another postdoctoral fellow in our laboratory, Dr. Lindsay Wieczorek, has been funded in part, by this U01 since September 2014. During this time she has worked closely with Drs. Sulik, Parnell and Fish on the Aim 1 studies. Additionally, she has attended the 2014 Neuroscience meeting, attended numerous UNC seminars, participated in CIFASD face to face meetings, and published the results of her studies regarding the HPA axis and behavioral dysfunction following early gestational ethanol exposure in mice. UNC-Chapel Hill has established the use of Individual Development Plans (IDPs) for postdoctoral fellows.

This includes an annual progress review/scholar evaluation directed toward identifying strengths and weaknesses and creating a roadmap for career advancement.

5. How have **results** been **disseminated** to communities of interest?

In addition to publications noted below, a number of scientific presentations have been made by our laboratory members, resulting in dissemination of our study results to communities of interest. Included are invited talks by Dr. Sulik and Dr. Parnell at the 6th International Conference on FASD, Vancouver Canada, March 2015, and a NOFAS webinar by Dr. Sulik entitled “Animal Model-Based Research: Insights Regarding Alcohol-Induced Abnormal Development” in August, 2014. Dr. Sulik also is a March 23rd, 2015 invited speaker at Ursinus College, Collegeville, PA, lecturing on "Embryos and Ethanol: Basic Research to Birth Defects Prevention", and lectured on embryology and FASD in January at the NIH main campus for a genetics course. Additionally, Drs. Wieczorek and Gilbert presented their research progress as part of the spring, 2015 UNC Bowles Center for Alcohol Studies seminar series.

6. What do you **plan to do for the next reporting period** (March 1, 2015 - April 1, 2016) to accomplish the goals?

- Prepare and submit a full paper on CP-55,940/alcohol co-exposure studies.
- Work with Mike Suttie to complete data processing and analyses for GD 10 and 11.5 exposures and publish the results of this study.
- Publish the results of our study examining Shh and Gli mutant mice following alcohol exposure on GD9.25.
- Present RSA abstracts in June 2015.
- Participate in a Gordon conference in May 2015.
- Assist Dr. Gilbert in developing and submitting a K99 proposal.

7. Describe your project’s **interrelation** with aims of the CIFASD consortium its other projects.

Our correlative brain/face studies complement those of Dr. Foroud’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.

8. **Publications** **[Accepted & In Press]**

Publications Reported for this Reporting Period

NIH Public Access Compliance	Citation
Complete	Parnell SE, Holloway HE, Baker LK, Styner MA, Sulik KK. Dysmorphogenic effects of first trimester-equivalent ethanol exposure in mice: a magnetic resonance microscopy-based study. Alcohol Clin Exp Res. 2014 Jul;38(7):2008-14. PubMed PMID: 24931007; PubMed Central PMCID: PMC4107075.
Complete	Cao W, Li W, Han H, O'Leary-Moore SK, Sulik KK, Allan Johnson G, Liu C. Prenatal alcohol exposure reduces magnetic susceptibility contrast and anisotropy in the white matter of mouse brains. Neuroimage. 2014 Nov 15;102 Pt 2:748-55. PubMed PMID: 25175539; PubMed Central PMCID: PMC4252734.
Complete	Lipinski RJ, Holloway HT, O'Leary-Moore SK, Ament JJ, Pecevich SJ, Cofer GP, Budin F, Everson JL, Johnson GA, Sulik KK. Characterization of subtle brain abnormalities in a mouse model of Hedgehog pathway antagonist-induced cleft lip and palate. PLoS One. 2014;9(7):e102603. PubMed PMID: 25047453; PubMed Central PMCID: PMC4105496.

Wieczorek LA, Fish EW, O'Leary-Moore SK, Parnell SE, Sulik KK. Hypothalamic-pituitary-adrenal axis and behavioral dysfunction following early binge-like prenatal alcohol exposure in mice. *Alcohol*; 2015, In press.

9. **Publications** **[In Preparation & Submitted]**

N/A

10. **Poster Abstracts and Presentations**

Abstracts

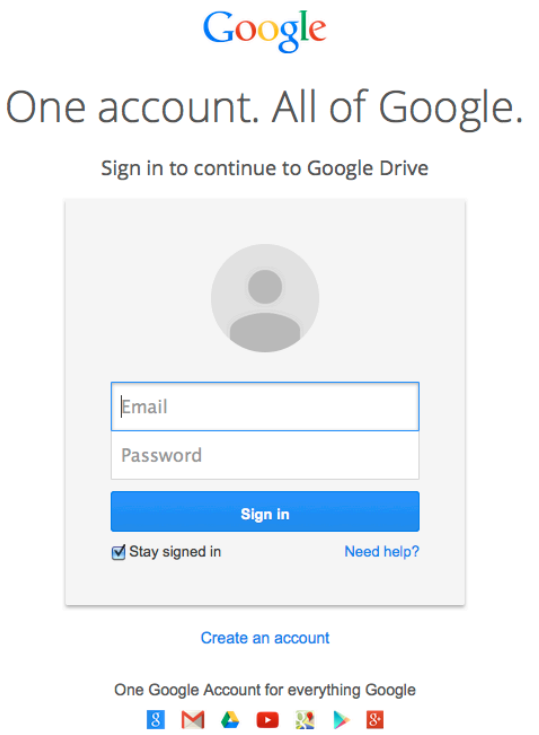
- Sulik KK, Gilbert MT, Wieczorek LA, Fish EW, Parnell SE. Alcohol Neuroteratogenicity – From basic science to Primary Prevention, AACAP 2014
- Gilbert MT, Parnell SE, Fish EW, Baker LK, Sulik KK. A mouse model shows synthetic cannabinoid teratogenicity and promise for prenatal drug co-exposure research. International Cannabinoid Research Society Meeting, Italy 2014
- L. Wieczorek, F. Budin, E. Fish, S Parnell, K. Sulik. 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,* 38:31A, 2014
- S.E, Parnell, L.K. Baker, K.K. Sulik. Early gestational ethanol exposure induces altered seizure susceptibility in neonatal and adolescent mice. *Alcohol Clin Exper Res*, 38:254A, 2014
- EW Fish, SE Parnell, MT Gilbert, KK Sulik, KP Williams. Sonic Hedgehog Pathway Agonist-Induced Birth Defects: Implications for Fetal Alcohol Spectrum Disorders. *Alcohol Clin Exper Res*, 38:35A, 2014
- Wieczorek L, Fish EW, Parnell SE, Sulik KK. Binge-Like Alcohol Exposure Early In Gestation Differentially Alters Stress Reactivity In Adult Male And Female Mice. Society for Neuroscience, 2014
- Gilbert MT, Parnell SE, Fish EW, Baker LK, Sulik KK A Mouse Model Of Early Prenatal Cannabinoid Exposure Shows Dose-Dependent Synthetic Cannabinoid Teratogenicity, Carolina Collaborative Cannabinoid Conference, Winston Salem NC, 2014

- Sulik KK, Wieczorek LA, Suttie M, Hammond P, Paniagua B, Budin F, Styner MA, Johnson GA, Parnell SE, Brain Imaging in FASD: Animal Studies, 6th International Conference on FASD, Vancouver Canada, March 2015
- Parnell SE, Fish EW, Gilbert MT, Wieczorek LA, Williams KP, Sulik KK, Murine-based studies of FASD susceptibility genes provide mechanistic data, 6th International Conference on FASD, Vancouver Canada, March 2015
- Fish EW, Sulik KK, Williams KP, Parnell SE. Vulnerability To Prenatal Alcohol-Induced Limb Defects: Haploinsufficiency In Sonic Hedgehog Pathway Genes, RSA submitted, 2015
- Fish EW and Parnell SE. Neurobehavioral Consequences Of Early Gestational Binge-Like Alcohol Exposure: Age-Related Alterations In Male And Female C57Bl/6J Mice RSA submitted 2015

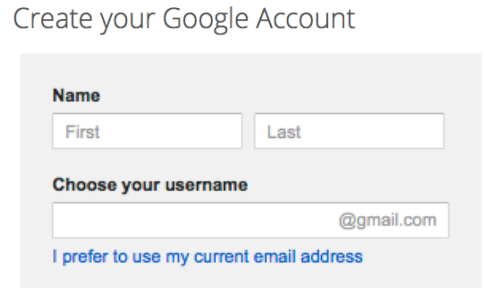
Presentations

In addition to platform and poster presentations at national and international meetings (abstracts above), Dr. Sulik presented a NOFAS webinar entitled “Animal Model-Based Research: Insights Regarding Alcohol-Induced Abnormal Development“ in August 2014, and is scheduled to give an invited lecture entitled “Embryos and Ethanol: Basic Research to Birth Defects Prevention” at Ursinus College, Collegeville PA, in March 2015. Additionally, Drs. Wieczorek and Gilbert are scheduled to present a UNC Bowles Center Seminar highlighting their postdoctoral research on March 23, 2015.

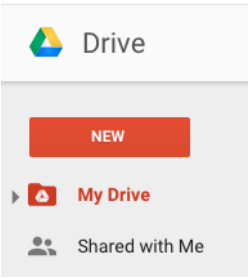
Google Drive
A lot of CIFASD information is shared via Google Drive.
SDSURF changed over to Google as our server and the file size we can send via email was reduced. Google is actually pretty great and makes it easy to share things with multiple viewers. Just type in Google Drive in your preferred browser to get started. Most use their daily email address as their login to their Google Account (i.e., I use vanderv@mail.sdsu.edu as the email I login with for Google).



Don't have a Google Account, no problem, click on 'Create an account.'
To use your current email address, click on 'I prefer to use my current email address' to establish your Google Account.



Once in Google, click on the triangle symbol for Google Drive or type in Google Drive again into your browser and then click on 'Shared with Me' to see the shared CIFASD files.



Any questions, just call me. Thanks. - Jill Vander Velde 619.594.4601

Ukraine Progress 2012-2017		
Ukraine Aims	Goal	Actual
Recruitment Exposed	60	88
Recruitment Comparison	60	74
2 YR Old Collections	240	117
3.5-4.5 YR Neurobehavior & Collections	240	116
Date of Update:	3/2/2015	# Fields

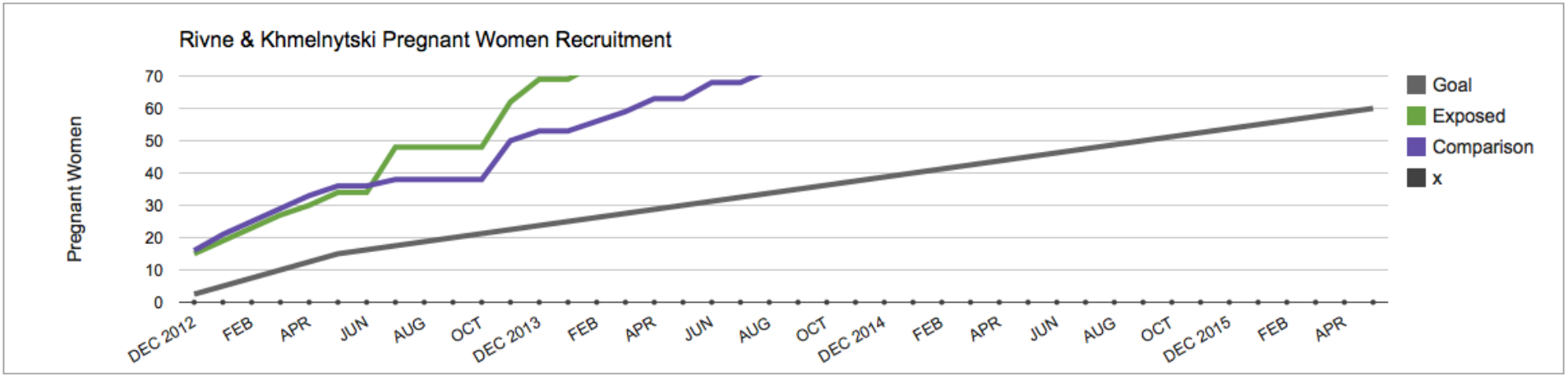
Phase III Goals (Cumulative)	Thru/By Date	Recruitment Exposed	Recruitment Comparison	2 YR Old Collections	3.5-4.5 YR Neurobehavior & Collections
Start YR1	8/10/2012	0	0	0	0
End YR1	5/31/2013	15	15	10	10
End YR2	5/31/2014	30	30	80	80
End YR3	5/31/2015	45	45	160	160
Data Collection End YR4	5/31/2016	60	60	240	240

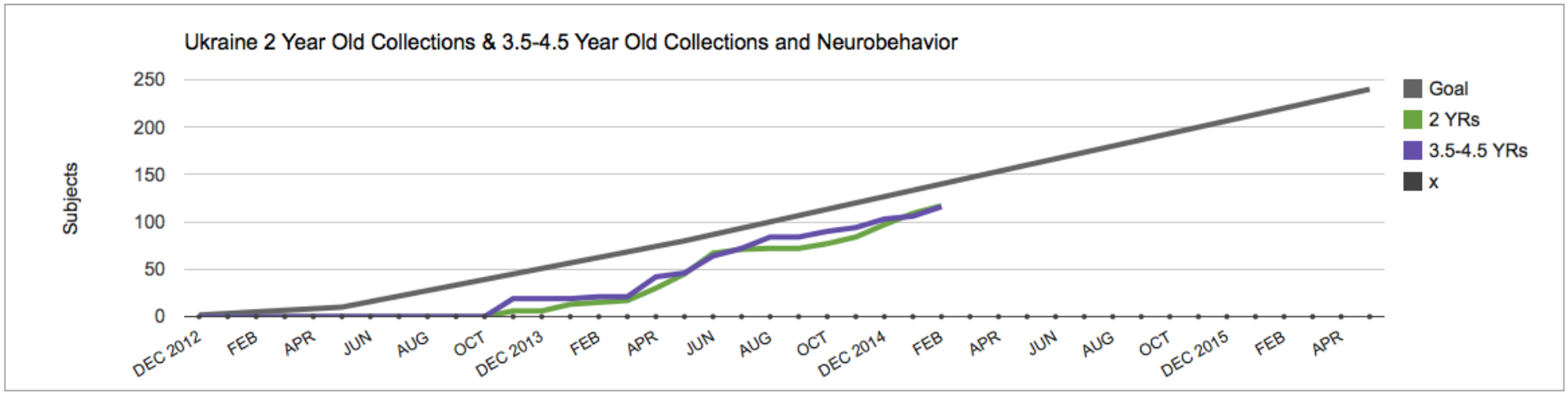
Project PI enters each month by the Monday before the Wednesday of the Conference Call by 9 AM Pacific.

Recruitment update: 5/30/14:
76 3D images collected; renewal of IRB going in this week to justify amending the new subject recruitment sample size increase from 120 to 200.

Ukraine Progress through February 2015

X in the keys that follow is to allow for a line to run along the x-axis indicating a hash mark (dot) per month.





3D Imaging and Saliva Collection Progress 2012-2017

3D Images	Goal	Actual
SDSU	264	162
UMN	264	204
Emory	264	152
USC	88	50
Ukraine	315	117
S. Africa PASS (Odendaal)	1080	1145
S. Africa (Jacobsons)	220	294
TOTAL	2495	2124

Saliva Samples	Goal	Actual
SDSU	264	142
UMN	264	190
Emory	264	234
USC	88	55
TOTAL	880	621

Date of Update:	2/27/2015	# Fields

3D Imaging 2012-2017

Phase III Goals Cumulative

	Thru/By Date	SDSU	UMN	Emory	USC	Ukraine	S. Africa PASS (Odendaal)	S. Africa (Jacobsons)
Start YR1	8/10/2012	0	0	0	0	0	0	0
End YR1	5/31/2013	30	30	30	10	10	900	0
End YR2	5/31/2014	96	96	96	32	95	1080	220
End YR3	5/31/2015	162	162	162	54	205	1080	220
End YR4	5/31/2016	228	228	228	76	315	1080	220
End YR5	5/31/2017	264	264	264	88	315	1080	220

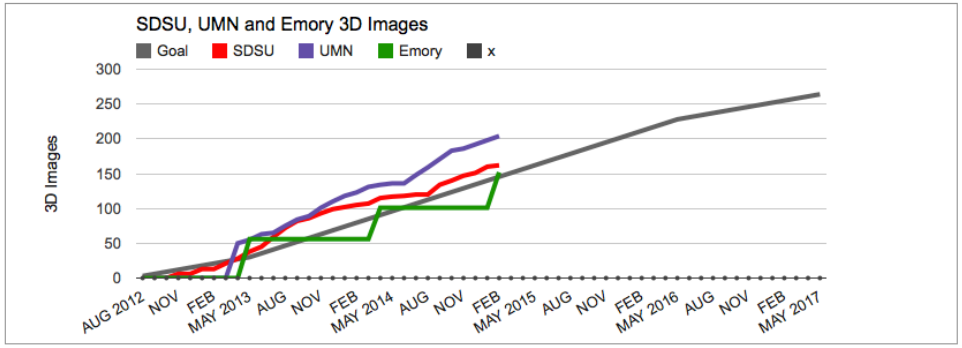
Saliva 2012-2017 (Phase III Goals Cumulative)

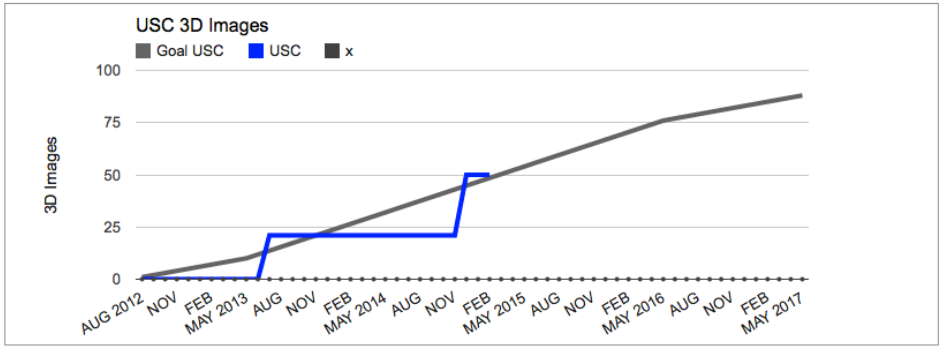
	Thru/By Date	SDSU	UMN	Emory	USC
Start YR1	8/10/2012	0	0	0	0
End YR1	5/31/2013	30	30	30	10
End YR2	5/31/2014	96	96	96	32
End YR3	5/31/2015	162	162	162	54
End YR4	5/31/2016	228	228	228	76
End YR5	5/31/2017	264	264	264	88

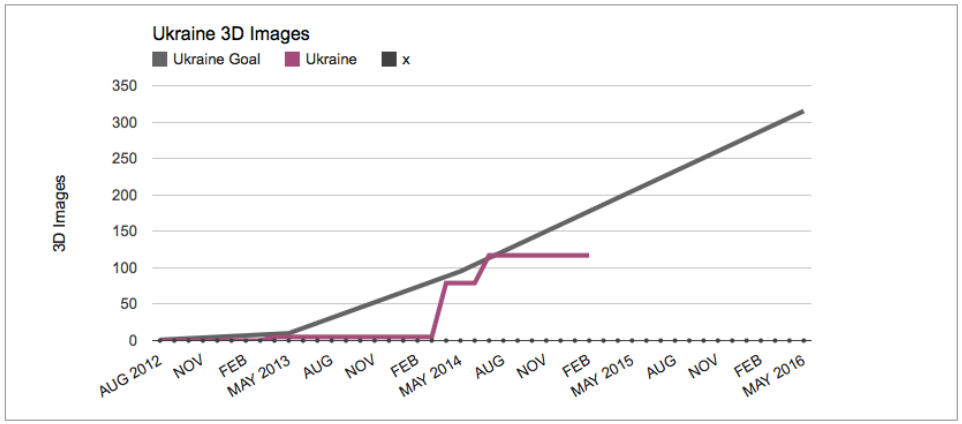
Project PI (or designated project staff) enters each month by the Monday before the Wednesday of the Conference Call by 9 AM Pacific.

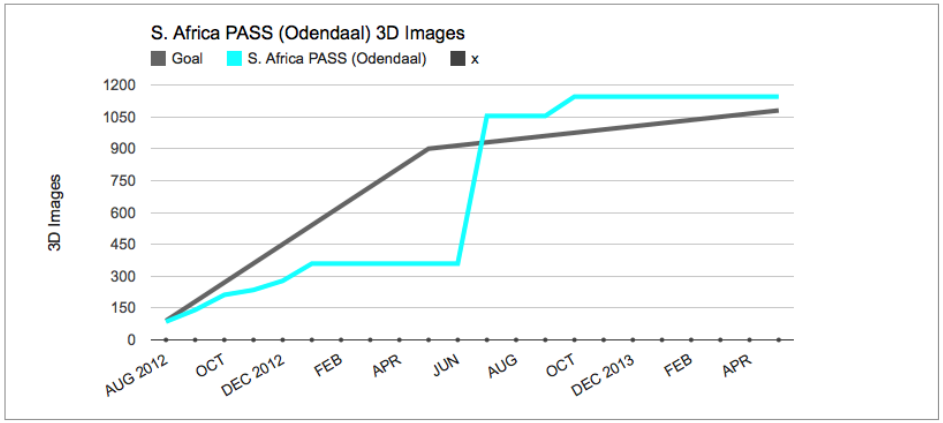
Explanation of modification to grant goals: Goals in this table reflect numbers provided by site PIs. Actual numbers reflect those for which an image has been received. Due to slow and inadequate internet service prohibiting electronic upload of files, two sites mail images on drives in batches therefore, monthly totals may not reflect the date the image was collected. Two sites do not have cameras and therefore schedule collection of images to coordinate with a clinic visit from Dr. Ken Jones. These sites will not produce continuous data month to month.

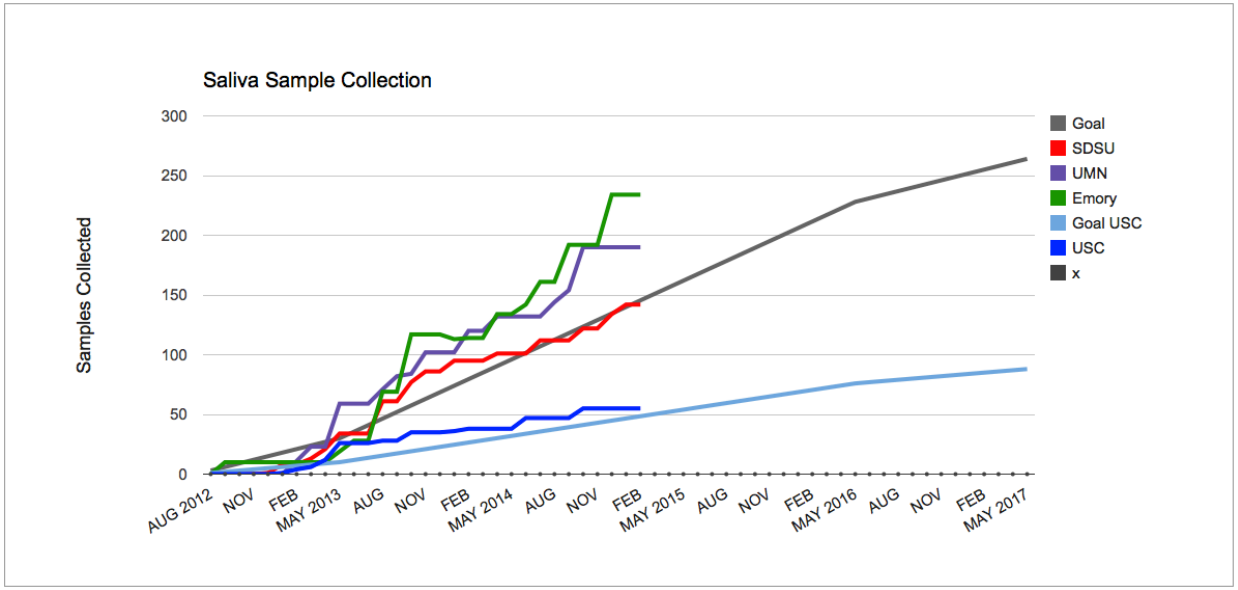
3D Images & Saliva Sample Collection thru February 2015 [294 3D Images at the South Africa (Jacobsons' site) were collected in Phase III.]











Neurobehavioral Progress 2012-2017

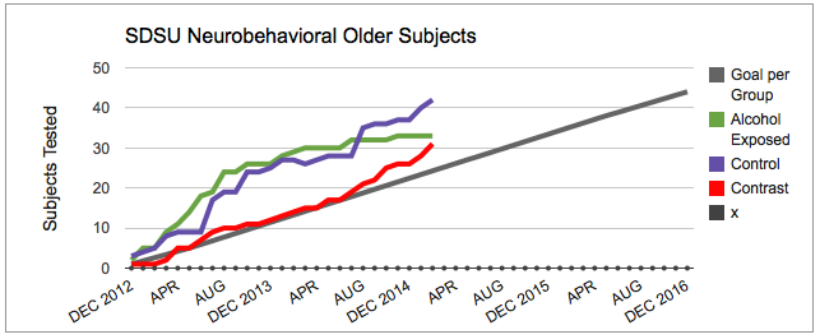
Site	Goal Per Group	Alcohol-Exposed	Control	Contrast
SDSU Old	44	33	42	31
SDSU Young	44	12	23	18
UMN Old	44	40	38	26
UMN Young	44	25	30	20
Emory Old	44	23	29	29
Emory Young	44	25	20	17
USC Old	44	17	19	n/a
	All Groups & Ages	Alcohol-Exposed	Control	Contrast
Tested Total	517	175	201	141
Goal Total	880	308	308	264
Date of Update:	3/2/2015	# Fields	Project PI (or designated project staff) enters each month by the Monday before the Wednesday of the Conference Call by 9 AM Pacific; #s are cumulative.	

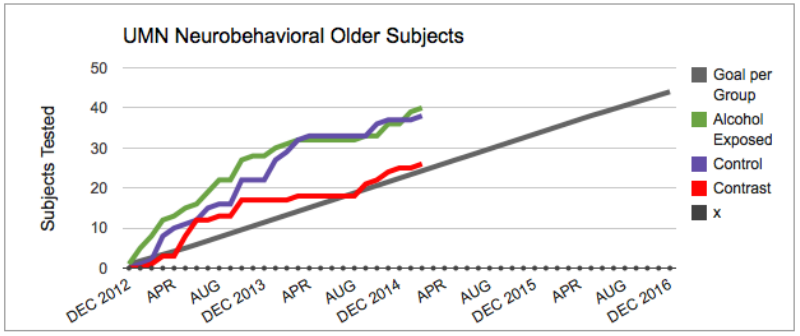
Site		SDSU						UMN						Emory						USC	
Goals (Cumulative)		Old			Young			Old			Young			Old			Young			Old	
Data Collection Subgroups	Thru/By Date	AE	CON	CT	AE	CON	CT	AE	CON	CT	AE	CON	CT	AE	CON	CT	AE	CON	CT	AE	CON
Start YR1	8/10/2012	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
End YR1	5/31/2013	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
End YR2	5/31/2014	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16
End YR3	5/31/2015	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27
End YR4	5/31/2016	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38
Data Collection End YR5	12/1/2016	44	44	44	44	44	44	44	44	44	44	44	44	44	44	44	44	44	44	44	44

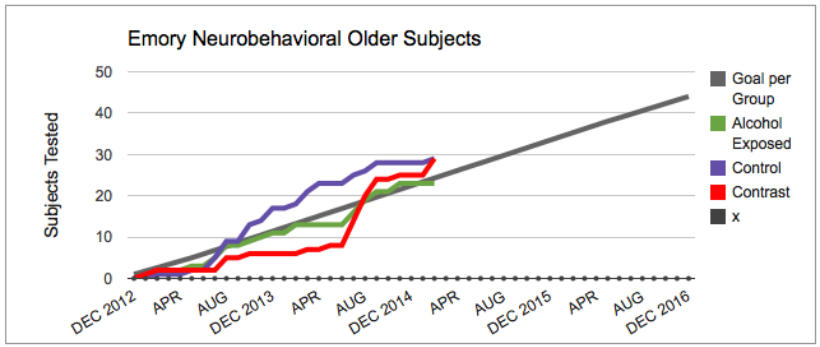
Total group goals across all sites for the older age study = 176 alcohol exposed, 176 control and 132 contrast for a total of 484.
Total group goals across all sites for the younger age study = 132 alcohol exposed, 132 control and 132 contrast for a total of 396. Both studies total = 880.

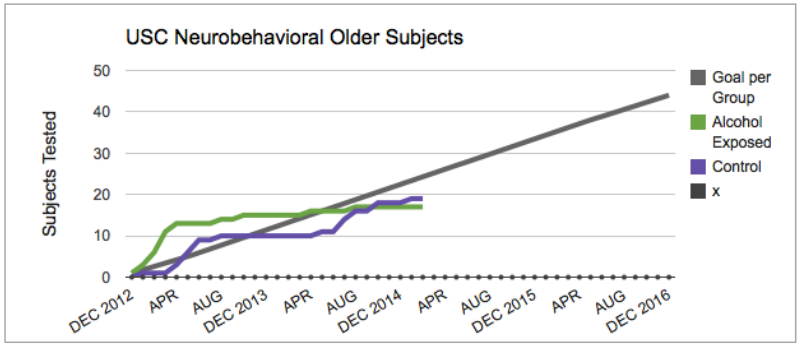
Explanation of Data Collection Goal Modification: Original proposal of 9 per year (5) per group (20) for a total of 900 (Grant Table 3) was incorrect; numbers should have followed the proposed timeline table in the grant. Overall numbers reduced by 2% to account for shortened first year and budget cuts.

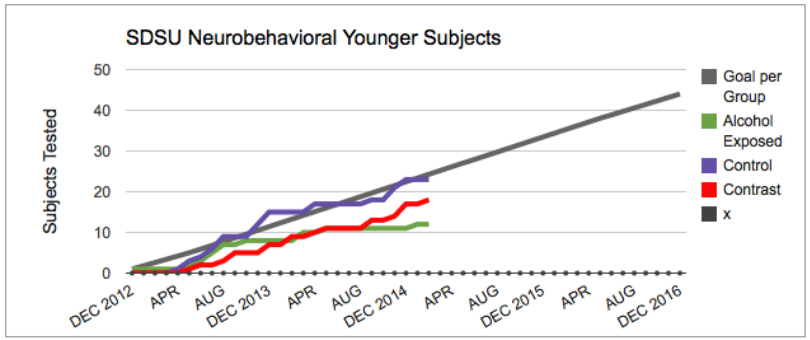
Neurobehavioral Subjects through February 2015

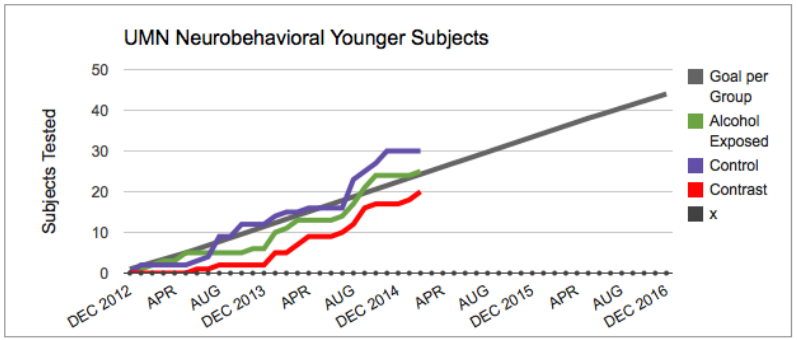


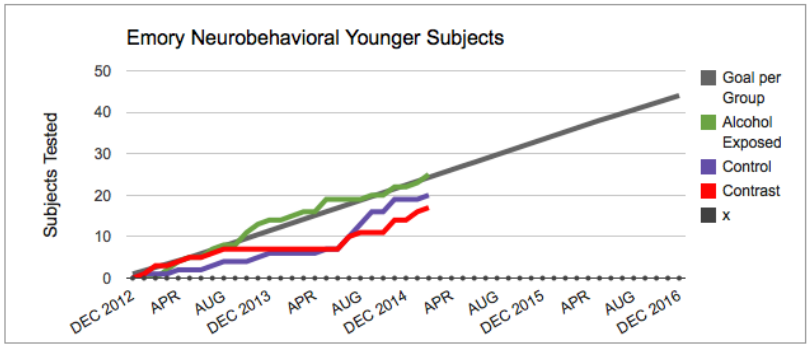












Neuroimaging Progress 2012 - 2017

Site	Time 1 Goal	Time 1 Scans	Time 2 Goal	Time 2 Scans
SDSU AE	30	26	30	0
SDSU CON	30	20	30	0
UMN AE	30	32	30	4
UMN CON	30	32	30	1
Emory AE	30	27	30	0
Emory CON	30	21	30	0
USC AE	30	32	30	4
USC CON	30	30	30	1
TOTAL	240	220	240	10

240 goal at each scan time = 120 AE and 120 CON

Date of Update: 3/3/2015 # Fields

Site		SDSU				UMN				Emory				USC			
Goals (Cumulative)		Time 1		Time 2		Time 1		Time 2		Time 1		Time 2		Time 1		Time 2	
Data Collection Subgroups	Thru/By Date	AE	CON	AE	CON	AE	CON	AE	CON	AE	CON	AE	CON	AE	CON	AE	CON
Start YR1	8/10/2012	0	0			0	0			0	0			0	0		
End YR1	5/31/2013	10	10			10	10			10	10			10	10		
End YR2	5/31/2014	20	20			20	20			20	20			20	20		
End YR3	5/31/2015	30	30	10	10	30	30	10	10	30	30	10	10	30	30	10	10
End YR4	5/31/2016			20	20			20	20			20	20			20	20
Data Collection End YR5	5/31/2017			30	30			30	30			30	30			30	30

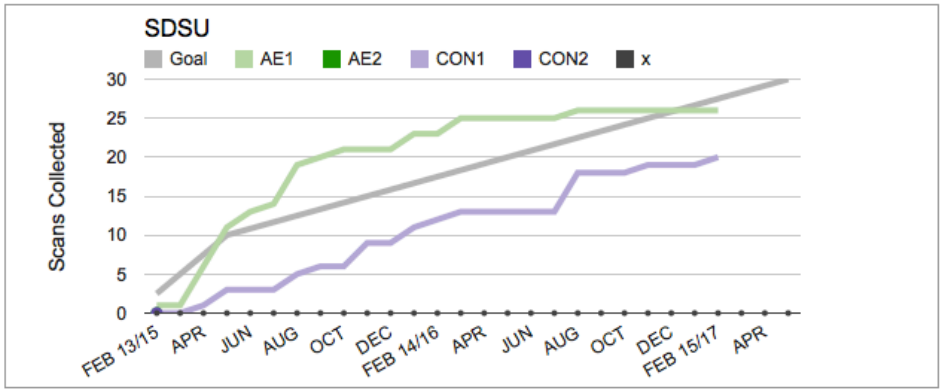
Each site will scan 30 AE and 30 CON two times for a total of 120 scan per site. Overall project unique subject goals are 120 AE and 120 CON for a total of 240 unique participants, therefore, matching the proposed grant totals.

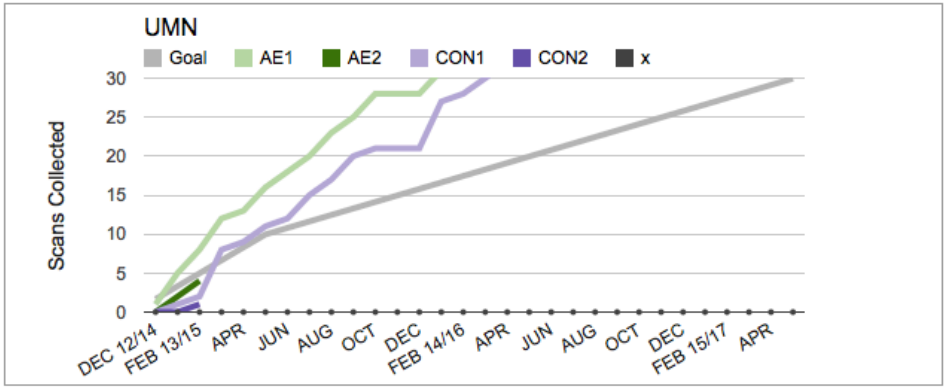
All Sites T1	AE	117	CON	103	All Sites T2	AE	8	CON	2	Time 2 Scan collection begins June 2014.

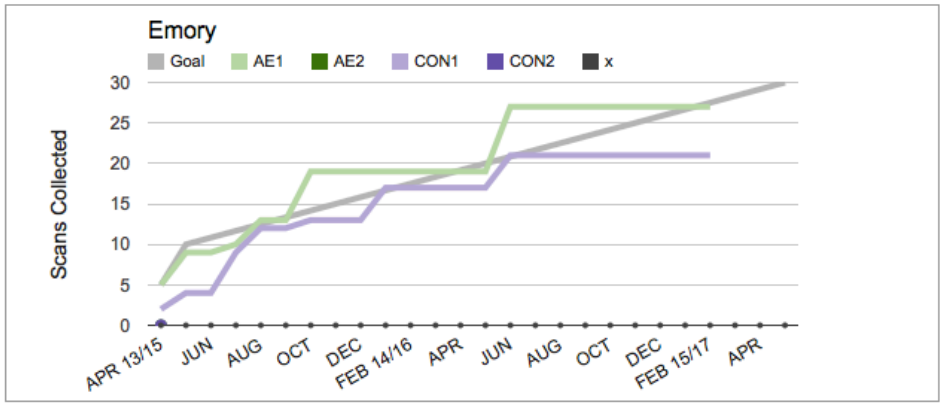
120 goal per group T1 AE, T1 CON, T2 AE, and T2 CON

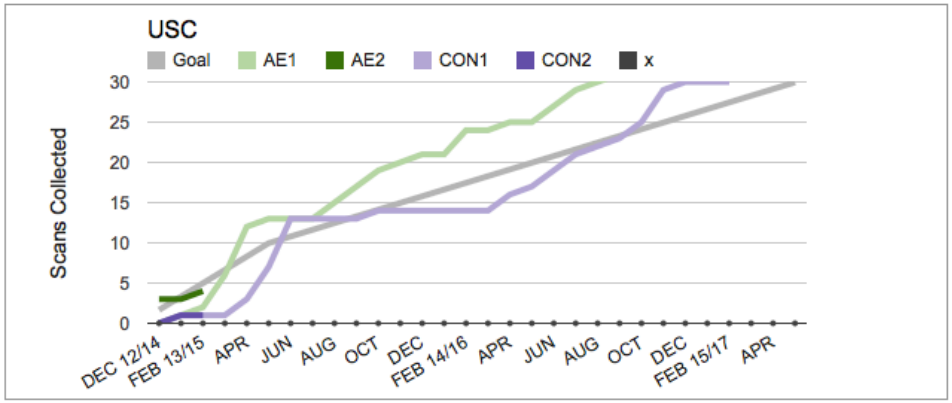
Project PI (or designated project staff) enters each month by the Monday before the Wednesday of the Conference Call by 9 AM Pacific; #s are cumulative.

Neuroimaging through February 2015
AE1 = Alcohol exposed subjects 1st scans. AE2 = Alcohol exposed subjects 2nd scans. CON = Control.









Percentages to Overall Phase III Goals

Neuroimaging

Percent to Overall Goals	SDSU	UMN	Emory	USC
Time 1 AE	87%	107%	90%	107%
Time 1 CON	67%	107%	70%	100%
Time 2 AE	0%	13%	0%	13%
Time 2 CON	0%	3%	0%	3%

Neurobehavioral

Old Percent to Goal	SDSU	UMN	Emory	USC
Alcohol-Exposed	75%	91%	52%	39%
Control	95%	86%	66%	43%
Contrast	70%	59%	66%	n/a
Young Percent to Goal	**SDSU**	**UMN**	**Emory**	
Alcohol-Exposed	27%	57%	57%	
Control	52%	68%	45%	
Contrast	41%	45%	39%	

Total NB Tested	
SDSU	159
UMN	179
Emory	143
USC	36
TOTAL	517

Ukraine

Ukraine Aims	% to Goal
Recruitment Exposed	147%
Recruitment Comparison	123%
2 YR Old Collections	49%
3.5-4.5 YR Neurobehavior & Collections	48%

3D Imaging & Saliva

3D Images	% to Goal
SDSU	61%
UMN	77%
Emory	58%
USC	57%
Ukraine	37%
S. Africa PASS (Odendaal)	106%
S. Africa (Jacobsons)	134%

Saliva Samples	% to Goal
SDSU	54%
UMN	72%
Emory	89%
USC	63%

Time into 5 Year Segment:	
2	Years
9	Months

CIFASD Publications

Not previously reported - CIFASD.org Publications (PubMed search)

- Balaraman S, Lunde ER, Sawant O, Cudd TA, Washburn SE, and Miranda RC. Maternal and neonatal plasma microRNA biomarkers for fetal alcohol exposure in an ovine model. *Alcoholism: Clinical and Experimental Research*, 2014 May;38(5):1390-400. PMCID:PMC3999266
- Cao W, Li W, Han H, O'Leary-Moore SK, Sulik KK, Allan Johnson G, and Liu C. Prenatal alcohol exposure reduces magnetic susceptibility contrast and anisotropy in the white matter of mouse brains. *Neuroimage*, 2014 Nov 15;102 Pt 2:748-55. PMCID:PMC4252734
- Ceccanti M, Fiorentino D, Coriale G, Kalberg WO, Buckley D, Hoyme HE, Gossage JP, Robinson LK, Manning M, Romeo M, Hasken JM, Tabachnick B, Blankenship J, and May PA. Maternal risk factors for fetal alcohol spectrum disorders in a province in Italy. *Drug Alcohol Depend.,* 2014 Dec 1;145:201-8.
- Chambers CD, Yevtushok L, Zymak-Zakutnya N, Korzhynskyy Y, Ostapchuk L, Akhmedzhanova D, Chan PH, Xu R, and Wertelecki W. Prevalence and predictors of maternal alcohol consumption in 2 regions of Ukraine. *Alcoholism: Clinical and Experimental Research*, 2014 Apr;38(4):1012-9. PMCID:PMC4024828
- Dou X and Charness ME. Effect of lipid raft disruption on ethanol inhibition of l1 adhesion. *Alcoholism: Clinical and Experimental Research*, 2014 Nov;38(11):2707-11. PMCID:PMC4278581
- Gautam P, Nuñez SC, Narr KL, Kan EC, and Sowell ER. Effects of prenatal alcohol exposure on the development of white matter volume and change in executive function. *Neuroimage Clin.,* 2014;5:19-27. PMCID:PMC4050317
- Gautam P, Nuñez SC, Narr KL, Mattson SN, May PA, Adnams CM, Riley EP, Jones KL, Kan EC, and Sowell ER. Developmental Trajectories for Visuo-Spatial Attention are Altered by Prenatal Alcohol Exposure: A Longitudinal FMRI Study. *Cerebral Cortex*, 2014 Aug 4.
- Hammond P, McKee S, Suttie M, Allanson J, Cobben JM, Maas SM, Quarrell O, Smith AC, Lewis S, Tassabehji M, Sisodiya S, Mattina T, and Hennekam R. Opposite effects on facial morphology due to gene dosage sensitivity. *Hum Genet.,* 2014 Sep;133(9):1117-25. PMCID:PMC4148161
- Hopman SM, Merks JH, Suttie M, Hennekam RC, and Hammond P. Face shape differs in phylogenetically related populations*. Eur J Hum Genet.,* 2014 Nov;22(11):1268-71. PMCID:PMC4200424
- Infante MA, Moore EM, Nguyen TT, Fourligas N, Mattson SN, and Riley EP. Objective assessment of ADHD core symptoms in children with heavy prenatal alcohol exposure. *Physiology and Behavior*, 2014 Oct 23.
- Lipinski RJ, Holloway HT, O'Leary-Moore SK, Ament JJ, Pecevich SJ, Cofer GP, Budin F, Everson JL, Johnson GA, and Sulik KK. Characterization of subtle brain abnormalities in a mouse model of Hedgehog pathway antagonist-induced cleft lip and palate. *PLoS One*, 2014;9(7):e102603. PMCID:PMC4105496
- Lovely CB, and Eberhart JK. Commentary: catching a conserved mechanism of ethanol teratogenicity. *Alcoholism: Clinical and Experimental Research*, 2014 Aug;38(8):2160-3. PMCID:PMC4147959
- Moore EM, Migliorini R, Infante MA, and Riley EP. Fetal Alcohol Spectrum Disorders: Recent Neuroimaging Findings. *Curr Dev Disord Rep.,* 2014 Sep;1(3):161-172. PMCID:PMC4207054
- Nguyen TT, Glass L, Coles CD, Kable JA, May PA, Kalberg WO, Sowell ER, Jones KL, Riley EP, Mattson SN, and the CIFASD. The clinical utility and specificity of parent report of executive function among children with prenatal alcohol exposure. *Journal of the International Neuropsychological Society*, 2014 Aug;20(7):704-16. PMCID:PMC4228981
- Parnell SE, Holloway HE, Baker LK, Styner MA, and Sulik KK. Dysmorphogenic effects of first trimester-equivalent ethanol exposure in mice: a magnetic resonance microscopy-based study. *Alcoholism: Clinical and Experimental Research*, 2014 Jul;38(7):2008-14. PMCID:PMC4107075
- Ware AL, Glass L, Crocker N, Deweese BN, Coles CD, Kable JA, May PA, Kalberg WO, Sowell ER, Jones KL, Riley EP, and Mattson SN, and the CIFASD. Effects of prenatal alcohol exposure and attention-deficit/hyperactivity disorder on adaptive functioning. *Alcoholism: Clinical and Experimental Research*, 2014 May;38(5):1439-47. PMCID:PMC3999227
- Wieczorek L, Fish EW, O'Leary-Moore SK, Parnell SE, and Sulik KK. Hypothalamic-pituitary-adrenal axis and behavioral dysfunction following early binge-like prenatal alcohol exposure in mice. *Alcohol*, 2015 Jan 26.