Spring 2016 Internal CIFASD Progress Reports
Austin, TX March 7-9, 2016

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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 AA014811-12

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. Progress in this area is essential if we are to have an efficient and effective means to identify children who are exposed to alcohol prenatally, so that interventions can be instituted to improve their long-term outcomes. Combining basic science and clinical projects within this larger collaboration, we can rapidly translate our findings both across species and potentially across domains to better understand the mechanisms whereby prenatal alcohol exposure disrupts development, and better recognize the range of outcomes encompassed by FASD.

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 believe our interdisciplinary, integrative approach creates a high probability of leading to improved and earlier diagnosis. 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. Such integration could only be accomplished with a multidisciplinary consortium. Our research projects utilize state-of-the-art techniques in brain imaging, craniofacial image analysis, behavioral phenotyping, and genetics. Our brain imaging project is expanding to examine functional connectivity, and will examine data longitudinally, as our preliminary data suggest differences in brain developmental trajectories between alcohol-exposed and control children. We are also proposing behavioral assessments at younger ages to create a clinically useful behavioral assessment battery, with good specificity and sensitivity, in hopes of identifying high-risk children so that interventions can be initiated as soon as possible. We recognize that differential diagnosis is important and therefore we will utilize a contrast group of clinically referred children with behavioral disorders or concerns to examine the specificity of our diagnosis. The use of 3-D facial image analysis with machine learning techniques may better identify the full range of FASD and the Dysmorphology Core now includes a telemedicine and training component to improve diagnostic capabilities at remote sites. We are also examining potential biomarkers of alcohol exposure and are examining how genetic and nutritional variables modify FASD to better identify individuals with, or at risk for, FASD. Such efforts should accelerate our progress in identifying children with prenatal alcohol exposure with the goal of early intervention.

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, with an additional new Educational Component that will help us disseminate the findings from the CIFASD.

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. Each month a CIFASD investigator presents their research to the other investigators. The Administrative Core continues to assist with the conferencing of smaller workgroups within CIFASD, such as recently with the cannabinoid use data group. The Administrative Core also maintains monthly updates on progress through the Google Drive spreadsheets and graphs, which are available to all CIFASD investigators and Advisory Committee members. Jennifer Thomas, this grant's Administrative Specialist, facilities contact between the consortium and the website designer to ensure the CIFASD.org website remains up-to-date.

Two new developmental projects were selected by the CIFASD Advisory Committee (Edward Riley, Michael Charness and Jennifer Thomas of the Administrative Core, the Science Advisory Board members and NIAAA staff) in the spring of 2015 and subcontracts were established and administered by the Administrative Core at SDSU Research Foundation to Joanne Weinberg at the University of British Columbia to work on identifying an immune signature characteristic of FASD and the implications for possible immune-based intervention strategies and to Kirsty Donald at the University of Cape Town to conduct a neuro- and 3D facial imaging study of two year-old children with alcohol exposure and controls embedded in a birth cohort study. These developmental projects are nearing completion of their first year of funding and will begin their second (and final) year of funding in June 2016. Both projects required State Department Clearance Requests as the sites are international; NIAAA staff were very helpful in facilitating these clearances. A third year of funding will begin for Tatiana Foroud's developmental project exploring genome-wide SNP genotyping FASD at Indiana in June as well. Two developmental projects wrapped up their third year of funding in May 2015, PIs: Johann Eberhart and Dipak Sarkar. Carryover funds received in October 2014 were extended to existing subcontracts to finish their developmental projects, PIs: Alison Noble and Rajesh Miranda, as well as an administrative supplement to Jeff Wozniak. Progress reports on the developmental projects, administrative supplement and the Educational Component of CIFASD, NOFAS, follow this report.

In May 2015, the Administrative Core was able to purchase equipment for CIFASD investigator Julie Kable to explore the aims proposed in her developmental project application which was scored and ranked among the top applications received; however, it was determined aims could be accomplished with an equipment only contribution from CIFASD. An update on this equipment supplement from the Administrative Core follows this report.

CIFASD investigators who attended the RSA meeting in San Antonio, TX in June 2015 convened for a luncheon meeting following the CIFASD symposium entitled, "The Face, Brain and Behavior in FASD: Connecting the Dots in CIFASD." Sara Jo Nixon was introduced as the newest member of the CIFASD Advisory Board, replacing Dr. Hannah Kinney, after accepting an invitation following the spring CIFASD face-to-face meeting held in March 2015 in Rockville, MD. Preparations are currently underway for the 2016 CIFASD face-to-face meeting which will be held in Austin, TX from March 7-9, 2016. The agenda will include discussion of the products from CIFASD Phase III goals and priorities and directions for the next phase of CIFASD.

As the next round of CIFASD's competitive grant renewals will likely be due in late 2016, Dr. Riley has been busy preparing for these submissions and evaluating the current projects. In June 2015, Dr. Riley requested each PI provide ideas on the direction their project could take in the next iteration of CIFASD; he also requested they identify the areas of FASD research they believe to be the most critical. In August 2015, each PI provided an update on the progress of each of their project's proposed aims and the deliverables that have resulted from their work to date as well as plans they have to accomplish their goals by May 2017. The reports were reviewed by the CIFASD Advisory Committee and summaries of their anonymous feedback were emailed to each PI in early November 2015.

Following acceptance of this grant's Federal Financial Report, a carryover request was submitted to NIAAA on November 2, 2015 to support Dr. Riley's increased effort and site visit travel in preparation of the competitive renewal, and to extend additional time to two existing developmental projects, PIs: Eberhart and Riley. Additionally, it was requested that carryover funds be used to support two additional new developmental projects: Susan Smith at the University of Wisconsin at Madison and Steven Youngentob at the University of Tennessee Health Science Center. Of the nearly 40 letters of intent received during the last call for developmental projects, roughly 15 were invited to submit more detailed proposals. Of those,

several were scored and ranked very highly by reviewers, both external and internal, and narrowing it down to the top two was a challenge for the Advisory Committee.

Key Outcomes. The submitted carryover request was just approved on February 19th, 2016. CIFASD results being shared in non-scientific news sources has been a big achievement during this reporting period. Importantly, new sources are seeking CIFASD investigators as experts for public interest pieces involving FASD. Coordinating and organizing the review of current CIFASD project progress and the ideas investigators have for Phase IV has been a key undertaking by the Administrative Core this year.

3. For this reporting period (April 1, 2015 - present), is there one or more **Revision/Supplement** associated with this award for which reporting is required? No. Information on the -13S1 Administrative Supplement (ND-PAE) was reported in last year's progress report; funding ended ended May 2015.

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

The CIFASD Educational Component, NOFAS, has continued to host their monthly webinar series which provides a platform for experts in the alcohol research field to reach a broad audience of viewers and listeners. The presentations are recorded and archived for further education and outreach. A schedule of upcoming webinars and links to previous webinar materials can be found online here: https://www.nofas.org/nofas-webinar-series/

Michael Charness, the Scientific Director of CIFASD, has organized two upcoming symposium highlighting CIFASD's research and accomplishments. The first was recently accepted for presentation at the upcoming 2016 RSA meeting to be held in New Orleans, LA in June. The symposium is entitled, "Genes, drugs, money, and vitamins: CIFASD studies of risk and resilience in FASD," and will include presentations by Johann Eberhart, Scott Parnell, Jeff Wozniak, and Tina Chambers, with Michael serving as the discussant. The session will promote CIFASD's research and illustrate the importance of genetics, cannabinoids, SES, and nutrition on clinical outcomes following prenatal alcohol exposure. Michael was also instrumental in helping the Informatics Core gain a slot to present at the 2016 FASD Study Group meeting and explain to the audience the CIFASD Data Sharing plan, process and tools which can allow external investigators access to CIFASD data. The second symposium Michael organized entitled, "Early identification of gestational alcohol exposure and fetal alcohol spectrum disorders in the CIFASD cohort," has been submitted for consideration at the upcoming ISBRA & ESBRA World Congress on Alcohol and Alcoholism to be held in Berlin, Germany in September. Presenters will include CIFASD investigators Rajesh Miranda, Dipak Sarkar, Julie Kable and Kirsty Donald; all researchers who have been recipients of developmental project funds from the Administrative Core.

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

Edward Riley, Michael Charness and Kathy Mitchell, as well as other CIFASD investigators, have been interviewed for recent pieces on FASD garnering international attention published by The Washington Post, SELF magazine, Harvard Gazette, and Four Corners, Australia's premier television current affairs program.

Details on the CIFASD Educational Component, NOFAS, which is tasked with disseminating CIFASD's results to various audiences can be found in their individual progress report, which follows this report.

6. Describe your project's **interrelation** with aims of the CIFASD consortium and other CIFASD projects. By its very nature, the Administrative Core interrelates with each of the CIFASD projects.

7. What do you **plan to do for the next reporting period** (March 1, 2016 - April 1, 2017) to accomplish your project's specific aims?

Beyond the regular oversight and management of CIFASD projects and their progress, the main task for the PI of the Administrative Core during the next reporting period will be to determine the charge and content of the next iteration of CIFASD and to help prepare the competitive renewal.

8. CIFASD Phase III ends in May of 2017. **What specific aims do you see your project addressing in the upcoming competitive renewal?**

The primary goal of the Administrative Core will be to facilitate the selection of research projects that address the needs of the RFA, should one be announced. The Administrative Core will help formulate the organization needed within CIFASD for the next phase to be competitive during the review process by assisting innovative projects to move forward, bringing new investigators into the field - if necessary, assisting in defining integration between projects, and compiling the record of achievements accomplished by CIFASD for the competitive renewal.

9. What do you believe the **hot areas of FASD research** to be?

Long-term consequences of prenatal alcohol exposure (e.g. health consequences in adults), innovative quick screening and diagnostic paradigms utilizing technology to relieve the personnel burden currently involved in these screening and diagnostic issues, and the search for biomarkers of exposures and/or biomarkers of resiliency or susceptibility to FASD.

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

The PubMed-generated publication list on our website now lists 177 publications total from CIFASD grants; 19 from April 2015 to present. Nine new papers cite the Administrative Core Grant, five of them with the AdminC PI as an author. Riley as an author:

NIH Public Access Compliance	Citation
Complete	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. 2015 Dec;25(12):4761-71. PubMed PMID: 25092900; PubMed Central PMCID: PMC4635917.
Complete	Moore EM, Riley EP. What Happens When Children with Fetal Alcohol Spectrum Disorders Become Adults?. Curr Dev Disord Rep. 2015 Sep;2(3):219-227. PubMed PMID: 26543794; NIHMSID: NIHMS702741; PubMed Central PMCID: PMC4629517.
Complete	Migliorini R, Moore EM, Glass L, Infante MA, Tapert SF, Jones KL, Mattson SN, Riley EP. Anterior cingulate cortex surface area relates to behavioral inhibition in adolescents with and without heavy prenatal alcohol exposure. Behav Brain Res. 2015 Oct 1;292:26-35. PubMed PMID: 26025509; NIHMSID: NIHMS705086; PubMed Central PMCID: PMC4558293.
Complete	Infante MA, Moore EM, Bischoff-Grethe A, Migliorini R, Mattson SN, Riley EP. Atypical cortical gyrification in adolescents with histories of heavy prenatal alcohol exposure. Brain Res. 2015 Oct 22;1624:446-54. PubMed PMID: 26275919; NIHMSID: NIHMS719411; PubMed Central PMCID: PMC4630133.
Complete	Murawski NJ, Moore EM, Thomas JD, Riley EP. Advances in Diagnosis and Treatment of Fetal Alcohol Spectrum Disorders: From Animal Models to Human Studies. Alcohol Res. 2015;37(1):97-108. PubMed PMID: 26259091; PubMed Central PMCID: PMC4476607.

U24 cited as a source of support on a developmental project publication:

NIH Public Access Compliance	Citation
Complete	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, May PA. Maternal risk factors for fetal alcohol spectrum disorders in a province in Italy. Drug Alcohol Depend. 2014 Dec 1;145:201-8. PubMed PMID: 25456331; NIHMSID: NIHMS754411; PubMed Central PMCID: PMC4736727.
Complete	Lee HS, Jones KL, Lee HK, Chambers CD. Fetal alcohol spectrum disorders: Clinical phenotype among a high-risk group of children and adolescents in Korea. Am J Med Genet A. 2016 Jan;170(1):19-23. PubMed PMID: 26384109; NIHMSID: NIHMS729778; PubMed Central PMCID: PMC4715586.
Complete	Vakhtin AA, Kodituwakku PW, Garcia CM, Tesche CD. Aberrant development of post-movement beta rebound in adolescents and young adults with fetal alcohol spectrum disorders. Neuroimage Clin. 2015;9:392-400. PubMed PMID: 26594621; PubMed Central PMCID: PMC4589820.

Confirmed with the author that the U24 was erroneously not included in the acknowledgments section of this paper and that is clearly associated with the U24 through developmental project funding:

NIH Public Access Compliance	Citation
Complete	Lovely CB, Nobles RD, Eberhart JK. Developmental age strengthens barriers to ethanol accumulation in zebrafish. Alcohol. 2014 Sep;48(6):595-602. PubMed PMID: 25012627; NIHMSID: NIHMS603452; PubMed Central PMCID: PMC4163099.

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

Publications in progress are mentioned in the individual developmental project progress reports which follow this report.

12. **Poster Abstracts and Presentations**

Edward Riley gave talks at the following conferences and meetings promoting CIFASD and its research during this reporting period:

"FASD: An Update on Recent Research and New Developments," keynote delivered at the 6th Annual Anishinabek G7 FASD Conference, Sault Ste. Marie, Ontario, Canada, October 2015.

"FASD: Alterations in Brain and Behavior Across the Life Span," presented at the FASD: Clinical Problem and Social meeting (WHO training), Krakow, Poland, September 2015.

"Fetal Alcohol Spectrum Disorders: An Overview and Update," plenary presentation as an invited speaker at the 15th Congress of the European Society for Biomedical Research on Alcoholism (ESBRA), also the 3rd Congress of the Latin American Society for Biomedical Research on Alcoholism (LASBRA), Valencia, Spain, September 2015.

"Fetal Alcohol Spectrum Disorder: Brain and Behavior," presented as an invited speaker at the 4th APSAAR (Asia-Pacific Society for Alcohol and Addiction Research) Congress, Sydney, Australia, August 2015.

“Healing the Broken Bough: Update on the Diagnosis, Prevention and Treatment of Fetal Alcohol Spectrum Disorders,” symposium presented at the 168th American Psychiatric Association Annual Meeting (Edward Riley served as the discussant and CIFASD investigators presented), Toronto, Canada, May 2015.

“Recent Research on FASD,” presented at Alcohol and Pregnancy: An Overview of Fetal Alcohol Spectrum Disorders, a Congressional Briefing sponsored by The Friends of NIAAA, Washington, D.C., April 2015.

“CIFASD Studies of Genetic Susceptibility to CIFASD,” a CIFASD plenary session presentation at the 6th International Conference on FASD: Research - Results and Relevance, Vancouver, BC, Canada, March 2015.

Principal Investigator(s): Tom Donaldson, 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, 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 in-person and on-line presentations to professionals and lay audiences, briefings for NOFAS affiliates and partners and—upon request—policymakers, and the incorporation and promotion of published findings in fact sheets, brochures and other awareness materials, curricula and other training materials, and press advisories and releases. 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 webinar series features CIFASD scientists and their research and 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 presentation was among those that were held during the reporting period:

- Jeff Wozniak, PhD – Treating FASD with Nutritional Interventions – March 2015

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

March 2015

- Lectured to the Physician Assistant Program, School of Medicine and Health Sciences, The George Washington University, Washington, D.C.
- Lectured to the Disease, Health and Promotion class at Georgetown University (pre-med and nursing students), Washington, D.C.

April 2015

- Organized and presented, along with other CIFASD members and Dr. George Koob, Director of NIAAA, at a Congressional Briefing on Capital Hill.
- Presented a webinar for the American Indian Association (AIA).
- Briefed aides to Senator Patty Murray and professional staff of the U.S. Senate Health, Education, Labor, and Pensions committee.

June 2015

- Presented a webinar for the Florida Alcohol and Drug Abuse Association.
- Briefed aides to Congressman Don Young and Mike Honda.
- Presented to 25 representatives of the NOFAS Affiliate Network at the annual affiliate summit.

September 2015

- Lectured to the Howard University School of Medicine, D.C.
- Presented a plenary at the University of New Mexico FASD Conference, Albuquerque, N.M.
- Presented to Congressman Tom Cole and his health staff.

October 2015

- Presented to the education and marketing committees of the National Alcohol Beverage Control Association (NABCA) at their annual meeting in Des Moines, IA.
- Presented a two-hour workshop at the Chesapeake Employee Assistance Practitioners Association (EAPA) quarterly meeting. Washington, D.C.
- Presented to the Montgomery County Chapter of the Rotary Club, Olney, MD.

November 2015

- Lectured to the School of Nursing Program at Georgetown University, Washington, D.C.
- Presented workshop at the *InRecovery* Magazine Expo and Gala, Prescott, AZ.

February 2016

- Presented a day long workshop for the Maryland Child Welfare Academy, Glen Burnie, MD.

In January 2016, NOFAS recommended Dr. Ken Jones be interviewed for a *Washington Post* article on NOFAS Vice President Kathy Mitchell. Dr. Jones was quoted in the article that reached over 7 million people and led FASD to trend on Facebook as the 6th most talked about topic in the days that followed publication of the article. NOFAS recommended CIFASD researchers, Dr. Michael Charness and Dr. Christina Chambers to media that wrote follow up articles. Both were featured in *Self Magazine*.

***NIAAA Twitter Chat** – NOFAS conducted a Twitter Chat in collaboration with NIAAA on September 9, 2015, International FASD Awareness Day.

***NOFAS International Gala** – Ken Jones spoke at the annual NOFAS Congressional event in September at the Embassy of France in Washington, D.C.

The **specific objectives** of the Educational Component of CIFASD are to:

- Promote CIFASD scientists and findings through webinars and other on-line platforms
- 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 linked to cifasd.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 access to state of the art resources and the opportunity for the best possible outcomes.

Key outcomes or other achievements. 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, 2015 - present), 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 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. Describe your project's **interrelation** with aims of the CIFASD consortium and other CIFASD 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.

7. What do you **plan to do for the next reporting period** (March 1, 2016 - April 1, 2017) to accomplish your project's specific aims? NOFAS will continue to include information on CIFASD research and accomplishments in a range of public presentations, meetings, and briefings. Public health and resource materials informed by CIFASD published findings will be developed and disseminated through the NOFAS Clearinghouse. NOFAS will also continue its webinar series and conduct outreach to the media and policymakers. NOFAS will also consider a national or international FASD conference or meeting that would in part highlight CIFASD research and its scientists.

8. CIFASD Phase III ends in May of 2017. **What specific aims do you see your project addressing in the upcoming competitive renewal?** Education and promotion of CIFASD findings, and linking the science to advances in the FASD knowledge base, public health messaging, diagnostic services, clinical interventions, and other tangible products and results.

9. What do you believe the **hot areas of FASD research** to be? Brain and behavior; neurocognitive function; genetic predisposition; epigenetic considerations; science with the highest probability to inform diagnostic criteria and guidelines, and clinical and pharmacological interventions.

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

Mitchell, K. (2015). What I wish I'd known about alcohol & pregnancy. *Healthy Mom&Baby, 2015*(18), 52–53.

Mitchell, K. (2015). A Ray of Sunshine. *inRecovery Magazine,* Fall 2015 (13), 22-23.

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

12. **Poster Abstracts and Presentations** Mentioned above.

Principal Investigator(s): Kirsten A. Donald
Co-investigators: Prof. Katherine Narr, Prof. Roger Woods, Shantanu Joshi Prof. Heather Zar, Prof. Colleen Adnams, Prof. Dan J. Stein
Institution(s): University of Cape Town, Health Sciences Faculty, Division of Developmental Pediatrics, Department of Paediatrics and Child Health and Department of Psychiatry and Mental Health.
University of California, Los Angeles, Health Sciences Faculty, Neurology department.
CIFASD Project Title: A neuro- and 3D facial imaging study of infants and 2-year old children with alcohol exposure compared to healthy unexposed controls embedded in a birth cohort study.
Grant Number: U24 AA014811 (Administrative Core of the CIFASD)

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

This proposed study will acquire high resolution structural, diffusion tensor (DTI) and resting blood- oxygen-level dependent (BOLD) functional imaging data and 3D facial photographs from a group of 2-year-old alcohol exposed and non-exposed children (n=50 per group respectively) in Cape Town, South Africa. These infants include a well characterized subsample of children enrolled in a Gates Foundation project, the Drakenstein Child Health Study, who have already been imaged as neonates using an ABMRF YI grant awarded to the PI. Leveraging the infrastructure of this large population study, the current proposal addresses novel hypotheses and integrates several advanced neuroimaging technologies together with functional (developmental and behavioral) outcomes.

The primary deliverable in this project will be a demonstration of how **non-invasive neuroimaging and 3D facial imaging may identify FASD-related brain abnormalities and/or dysmorphology before symptoms of FASD are diagnosed.**

The proposed study will also monitor **longitudinal effects of alcohol-exposure on the developing brain structure in children from birth through early childhood**, which have not been previously addressed across this early developmental period in humans. This project will also demonstrate feasibility of this technology and approach in a low-income setting where the burden of these disorders is exceptionally high and need for prevention and intervention is critical.

Study design and staff. As this study successfully uses the infrastructure setup for the Drakenstein Child Health Study (DCHS), many operational costs of this nested sub-study are absorbed by the umbrella study. For example, the extensive network of staff from the umbrella study, which are involved in this nested sub-study as well, are not paid from this grant, therefore these costs are absorbed by the umbrella study. The core DCHS has retained 32 initial and additional research staff (which includes 12 members of nursing staff), which were recruited and trained for this study.

However, additional staff were recruited for tasks specific to this sub-study such as the employment of a proportion of a post-doc to assist with preliminary data cleaning and imaging analysis.

Recruitment and enrolment in the DCHS. Recruitment of participants is completed. We currently have recruited 1140 mother-infant pairs in the umbrella study. The last infant to be born was in September 2015.

Investigations and data collection of data with specific relevance to this project:

Imaging: Neuroimaging and 3D facial photography of infants is ongoing.

Developmental assessments: Administering of Bayley Scales of Infant Development III (BSID III) assessments is ongoing.

Data entry and analysis: Preliminary findings have been published and data analysis is ongoing.

Timeline. The projected umbrella study duration is 9 years (All children to be followed up to age 5 years). *Entry point for this proposal was at year 4 (quarter 3 and 4).*

Year 1: (Complete): In the first year, recruitment of study stuff and of the pilot participants took place. Site preparation was completed and detailed study documentation developed by the study team.

Year 2: (Complete), the majority of infants were born, and had early neuro-behavioral assessments and neuroimaging. Commenced 6-month developmental assessments.

Year 3: (Complete 2013-2014): Ongoing 6-month developmental assessments. Commence 24-month developmental and behavioural assessments. Preliminary data analysis commences.

Year 4: (Complete: 2014-2015): Ongoing 6-month and 24-month developmental and behavioral assessments. Preliminary data analysis on neonatal neuroimaging complete, published and disseminated results through publication and presentations. **Commenced 24-month photography and neuroimaging.**

Year 5: (Current: 2015-2016): Complete 6-month and continue 24-month developmental and behavioral assessments and 24-month neuroimaging.

Year 6: (2016-2017) Complete 24-month developmental and behavioral assessments and 24-month neuroimaging. Commence formal data analysis, manuscript preparation and disseminate results through publication and presentations.

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

The focus of this report is on current research activities as this developmental grant is in its first year. However, I also report relevant preliminary results on the earlier age group imaged prior to this study and which are relevant to its goals and progress.

Major Activities:

Brain-imaging and photography of infants and 2-year old children. Neuroimaging of infants is ongoing. 236 two-to-six-week olds and 26 two-year olds, have already been imaged as at the end of January 2016. Currently imaging 2 year olds at a rate of 1-2 a week. This is in line with the original proposal which aimed to have completed imaging on half the cohort (50 infants), by mid-year 2016.

Developmental assessments of infants and 2-year old children. Bayley Scales of Infant and Toddler development (BSID III) are ongoing. 216 Bayley's assessments have been completed at 6-months, and 283 have been completed at 24-months (the number of children having BSID III includes, but exceeds this sub-study cohort).

Community development. This is not a specifically defined goal of this project. However, nurses and community health workers in state service at the community clinics (in addition to the research study staff) where recruitment and follow up for the DCHS occur, have been trained in developmental screening and referral by our team as part of our community support in early identification of developmental disabilities in this high risk community.

Published outputs from project. Research outputs generated from this project under items 10, 11, and 12.

Preliminary findings from the neonatal imaging that precedes this grant but documents the same cohort we are currently investigating. Putting the data together there is evidence for gray matter volume differences, differences in the integrity of white matter microstructure in white matter tracts which connect to both these areas, abnormal glutamate/glutamine concentrations in the parietal lobe as well as differences in the organization of functional networks for a small group of these infants. Together this provides evidence for the effect of prenatal alcohol exposure on the brain at a structural, microstructural, neurochemical as well as functional organizational level in the *same group of infants*. This has not been previously reported. These alterations suggest prenatal alcohol exposure affects multiple biological processes that result in physical growth of the brain (overall as well as regional volume changes), quality and maturation of the connecting circuitry (white matter miscrostructural metrics), neurotransmitter concentrations in the first weeks of life (glutamatergic function) as well as functional connectivity, both within intrinsic networks as well as between hemispheres. Although some of these effects may represent delayed maturation, the coherance of the described regions with previously described findings in older children as well as in our understanding of the functional deficits we expect to see in children on the FASD spectrum argues against this as the only explanation. The regions and connecting circuitry described here are likely to be at least part of the *primary core* effect of alcohol exposure on the developing brain.

It is important to note that analyses were conducted in these infants before any formal diagnosis could be made and thus effect sizes are expected to be smaller. This is true of all the analyses described here, the changes described in the publications documented below represent the group effects of infants with moderate-severe alcohol exposure at any stage in the prenatal period. This was a potentially risky strategy to adopt for defining the alcohol-exposed group (against describing the severe end of the exposure spectrum). However, based on the previous literature in the imaging and developmental psychology fields, I believed that there was evidence for the harmful effects of *any* prenatal alcohol on the developing brain. The combined data which is described in this these papers provides support for this hypothesis.

Further evidence for this effect are the widespread correlations between developmental outcomes at 6 months and gray matter volume at birth. These data underscore the fact that the harmful effects of moderate-severe prenatal exposure to alcohol on brain development at neurobiological and clinical level are already discernible in the first months of life, well before the age FAS and FASD are typically diagnosed. Although the use of the newer quantitative MRI techniques are not yet easily translatable into use in individual identification (these reports represent between group effects), this is something that could be developed. Approaches such as identifying “cut-off” values/ranges for any of the different MRI modalities described at a specific age, outside of which exposed children could be considered at risk, especially if they had additional clinical concerns may be avenues for adding the huge bulk of imaging information the research community has acquired over the last 25 years, to pathways to care. However, to do this effectively, large collaborative analyses using data from multiple populations will be necessary and data collected across understudied time-points, especially the early years.

3. For this reporting period (April 1, 2015 - present), 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?

Mentorship and skills development of postdoctoral fellow. Fellow drafted and wrote a review paper (First author): du Plooy, C. P., Malcolm-Smith, S., Adnams, C. M., Stein, D. J., & Donald, K. A. The effects of prenatal alcohol exposure on episodic memory functioning: A systematic review. *Archives of Clinical Neuropsychology*. In review.

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

Initial results have been fed back to lay audiences (community centre) as well as to local psychiatry and pediatric staff working in the public sector in the region in November 2015.

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

Neuroimaging studies have indicated that prenatal alcohol exposure is associated with alterations in the structure of specific brain regions. Indeed, recent work from the NIAAA sponsored Collaborative Initiative on Fetal Alcohol Spec- trum Disorders (CIFASD) has established that prenatal alcohol exposure affects the volumes of specific brain regions in different ways at different time points in older children. At 5-6 years, alcohol exposed children demonstrated larger cortical volumes (particularly in the parietal cortex) compared to unexposed control subjects. Over time these regions in alcohol-exposed children showed a linear drop-off in volume with ultimately lower volumes in adolescence than control children. This suggests reduced efficiency and plasticity in brain development. However, information on the critical infant years when the greatest changes in brain volumes occur has remained a largely missing piece in understanding the initial effects of prenatal alcohol exposures until now**.** Imaging neonates safely and successfully is challenging. This study represents one of the first and largest cohorts at this critical age investigating the neurobiological effects of prenatal alcohol exposure.

This research project aims to show how sophisticated brain-imaging techniques (and 3D-facial imaging) may be able to identify early FASD-related abnormalities with the brain, before developmental and behavioural problems are observed. This research project also looks at the long-term effects of alcohol-exposure on a young child's developing brain from birth to 2-years of age.

As previous research has established, drinking alcohol during pregnancy can result in harmful consequences for the unborn child. This '*in utero*' exposure to alcohol can physically alter regions of the child's brain, which, as established by our previous research, can already be observed in newborns. The question following this is whether these early brain abnormalities can predict developmental and behavioural problems in later childhood.

During this period, from birth to 2-years of age, higher-level brain networks have not yet been established and outside environmental influences have not yet made a significant impact on the developing brain. The brain has a high degree of plasticity at this time, and is developing rapidly, with significant changes occurring in the structural and functional networks of the brain. Making this both a vulnerable and very important time in the development of the brain.

7. What do you **plan to do for the next reporting period** (March 1, 2016 - April 1, 2017) to accomplish your project's specific aims?

- Ongoing neuroimaging and photography of infants at 2 years old.
- Ongoing BSID III assessments at 6-months, and 24-months old.
    - Completion of formal data analysis.
- Preparation of manuscript for publication
- Secure further funding for additional imaging time points and epigenetic investigations

8. CIFASD Phase III ends in May of 2017. **What specific aims could you see your project addressing in the upcoming competitive renewal?**

- Consolidation of neuroimaging in the early years (<6 years) by additional time point for this cohort.

- Investigation of the genetic and epigenetic associations with structural and functional neuroimaging and facial features in this population.

9. What do you believe the **hot areas of FASD research** to be?

Expanding the understanding of how exposure to neurotoxic substances during fetal life and subsequent early development leads to permanent structural and plasticity deficits is clinically relevant. Critical future focus areas that are needed to begin to explain the variation in clinical phenotype of children with prenatal alcohol exposure and hence risk, include the following: the investigation of hypothesis driven candidate gene expression/ alterations (with the SRC/Fyn gene being a promising current candidate in cohorts with European ancestry), epigentic modification of genetic code in prenatal alcohol exposure both at an individual and intergenerational level which may explain the gene-environment interactions which we know exist. Studies which explore these critical areas and relate them to both clinical, neuroimaging and facial morphological outcomes are likely to provide a deeper understanding of the mechanisms for vulnerability to the effects of prenatal alcohol exposure and in so doing providing potential directed targets for biological interventions to ameliorate the effects of prenatal alcohol on subsequent brain development and functional outcomes when prevention fails. There is a need for a better understanding of the underlying mechanisms of development if we want to pro- mote optimal development, not only in terms of cognition, but also in reducing risk for later alcohol use disorders in these children as they emerge into adolescence.
The other key remaining question is that of the meaning of these findings in the context of these children's later brain development both at a neuroanatomical and functional level. Documenting the trajectory over time, may further address gaps in our understanding of which areas and networks have key functional importance for this group of children.

An independent study has reported volume changes in prenatal alcohol exposed infants using tensor-based morphometry analysis. These findings were most prominent in cerebellum, striatal and frontal midline areas. The location of these results is remarkably consistent with an established theme in prenatal alcohol exposure studies of the importance of midline structures. We expect that the identification of alcohol effects on key midline structures in the infant brain may be an early marker for later functional cognitive and behavioral deficits. However, only longitudinal imaging, developmental and behavioral data will enable us to confirm the clinical significance of very early identification of these children. Further, our preliminary resting state functional MRI analysis demonstrated significant differences in the motor network in neonates with prenatal alcohol exposure and suggests that alcohol exposure disrupts the temporal coherence in blood oxygen utilization across the brain at rest. This highly sensitive approach may prove to be valuable in identifying affected children early as well as monitoring the effectiveness of interventions. A longitudinal approach may consolidate these early findings by the addition of later time points and establish relationships between structural and functional imaging findings and clinical measures including not only formal cognitive or develop- mental and behavioral assessments, but also more integrative outcomes such as academic achievement in real-world settings.

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

Below are the publications and conference presentations which relate directly to the prenatal alcohol exposure infant imaging project and are on the *same cohort* which we are imaging and photographing at 2 years for the CIFASD developmental grant. These publications do not acknowledge CIFASD because they weren't funded by this grant, but I list them in this report because they relate to the aims and progress on the project overall and represent important data on which the longitudinal analysis in the developmental CIFASD grant will build.

NIH Public Access Compliance	Citation
Not applicable	Donald KA, Fouche JP, Roos A, Koen N, Howells FM, Riley EP, Woods RP, Zar HJ, Narr KL, Stein DJ. Alcohol exposure in utero is associated with decreased gray matter volume in neonates. Metab Brain Dis. 2016 Feb;31(1):81-91. PubMed PMID: 26616173.
Not applicable	Donald KA, Ipser JC, Howells FM, Roos A, Fouche JP, Riley EP, Koen N, Woods RP, Biswal B, Zar HJ, Narr KL, Stein DJ. Interhemispheric Functional Brain Connectivity in Neonates with Prenatal Alcohol Exposure: Preliminary Findings. Alcohol Clin Exp Res. 2016 Jan;40(1):113-21. PubMed PMID: 26727529.
Not applicable	Donald KA, Roos A, Fouche JP, Koen N, Howells FM, Woods RP, Zar HJ, Narr KL, Stein DJ. A study of the effects of prenatal alcohol exposure on white matter microstructural integrity at birth. Acta Neuropsychiatr. 2015 Aug;27(4):197-205. PubMed PMID: 26022619.
Not applicable	Donald KA, Eastman E, Howells FM, Adnams C, Riley EP, Woods RP, Narr KL, Stein DJ. Neuroimaging effects of prenatal alcohol exposure on the developing human brain: a magnetic resonance imaging review. Acta Neuropsychiatr. 2015 Oct;27(5):251-69. PubMed PMID: 25780875.

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

du Plooy, C. P., Malcolm-Smith, S., Adnams, C. M., Stein, D. J., & Donald, K. A. The effects of prenatal alcohol exposure on episodic memory functioning: A systematic review. *Archives of Clinical Neuropsychology*. In review

Howells, FM; Donald, KA; Roos, A; Woods, RP; Zar, HJ; Narr, KL; Stein, DJ. Reduced glutamate in white matter marks prenatal exposure to alcohol in neonates: A cross- sectional ^{1}H-MRS study. *Metabolic Brain Disease*. In review.

KA Donald, AC Fernandez, K Claborn, C Kuo, N Koen, H Zar, DJ Stein: The Effects Of HIV And Alcohol On Birth Outcomes In South Africa. *BMC Pediatrics.* In review

12. **Poster Abstracts and Presentations**

KA Donald, AC Fernandez, K Claborn, C Kuo, N Koen, H Zar, DJ Stein: The Effects Of HIV And Alcohol On Birth Outcomes In South Africa. *Alcoholism: Clinical and Experimental Research* Volume 39, s1, June 2015. Published conference proceedings, 38th Annual Scientific Meeting of the Research Society on Alcoholism (RSA), San Antonio, Texas.

KA Donald, J Ipser, FM Howells, A Roos, J-P Fouche, EP Riley, N Koen, RP Woods, B Biswal, HJ Zar, KL Narr & DJ Stein. Inter-Hemispheric Functional Brain Connectivity In Neonates With Prenatal Alcohol Exposure. *Alcoholism: Clinical and Experimental Research*. Volume 39, s1, June 2015. Published conference proceedings, 38th Annual Scientific Meeting of the Research Society on Alcoholism (RSA), San Antonio, Texas. *Tim Cudd award presentation FASDG Study day.*

KA Donald, F Howells, A Roos, K Narr, R Woods & D Stein. Alcohol Exposure Effects In Infants: Neonatal Magnetic Resonance Spectroscopy Findings. *Alcoholism: Clinical and Experimental Research*. Volume 37, s2, June 2013. Published conference proceedings, 36th Annual Scientific Meeting of the Research Society on Alcoholism (RSA), Orlando, Florida.

Principal Investigator(s): Joanne Weinberg
Co-I: Charlis Raineki; PhD student/PDF: Tamara Bodnar
Institution(s): University of British Columbia
CIFASD Project Title: Identifying an immune signature characteristic of FASD: Implications for possible immune-based intervention strategies
Grant Number: U24 AA014811 (Administrative Core of the CIFASD)

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

This CIFASD Developmental Project was inspired by data from our animal model work on neuroimmune function, which demonstrated that: 1) Following an immune/inflammatory challenge, in adulthood, animals prenatally exposed to alcohol exhibit a more severe and prolonged course of inflammation; and 2) This exacerbated response may have its basis in a prenatal alcohol-induced pro-inflammatory bias that is present from early life. It is not known whether children with FASD exhibit a similar pro-inflammatory profile. However, evidence is accumulating to suggest that this, indeed, may be the case. In this context, we are collaborating with Dr. Christina Chambers on her U01 to analyze samples from pregnant women and their children in the Ukraine cohorts. The Specific Aims are to:

Probe for a unique immune signature (early pro-inflammatory bias?) in children with FASD that may be linked to maternal immune function

- Assay plasma samples from pregnant women for cytokine and C-reactive protein (CRP) expression
- Assess both plasma and salivary markers in their children. This will allow us to determine if there are alcohol-specific alterations in saliva that parallel those in blood
- Measure endocrine (cortisol) function in parallel to provide an index of endocrine-immune interaction
- Investigate whether nutritional supplementation can modulate the expected pro-inflammatory/endocrine profiles in both mothers and children

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

Please note: Our project award dates for Year 1 are June 1, 2015 - May 31, 2016. The subaward account was established at University of British Columbia on November 17, 2015. Due to extensive preparation and planning for this project in collaboration with Dr. Tina Chambers and her team throughout the summer and fall, we were able to begin processing samples within two weeks of having access to the funds. We believe that we have made significant progress toward achieving our Year 1 goals.

Major activities. We began by analyzing plasma samples from the pregnant women. Samples that had been shipped to UC Davis were shipped back to UCSD and from UCSD to UBC. With help from Dr. Chambers, Bette Cesna and Jordan Schafer, all available samples were sorted and categorized by outcomes of the children: A: Alcohol-consuming women whose children were affected (facial features, growth, neurobehavioral deficits), n=36; B: Alcohol-consuming women whose children were not affected (no features, no growth or neurobehavioral deficits), n=22; and C: Women with low-no alcohol consumption, n=94. Two samples from each subject, one taken at enrollment (~second trimester) and the second at ~ 32 wk gestation (third trimester) were assayed. Samples were analyzed using the Human Biomarker 40-Plex Kit from MesoScale Discovery, which has a wide dynamic range and the best available assay sensitivity. Pro- and anti-inflammatory cytokines and chemokines, as well as angiogenesis and vascular injury markers, and CRP, a general marker of inflammation, have been assayed to date.

Specific objectives. Our objective for Year 1 was to focus on analysis of plasma samples from the pregnant women, to assay for cytokine and C-reactive protein (CRP) expression, as well as endocrine (cortisol) function, to provide an index of endocrine-immune interaction.

Significant results and key outcomes. As background for our results: Cytokines are generally categorized as pro-inflammatory (cell-mediated or Th1-type immunity) or anti-inflammatory (humoral or Th2-type immunity). Inflammation is upregulated following activation of Th1 cells, whereas Th2 cells play a role in downregulating Th1 pro-inflammatory responses in response to an increase in Th2 cytokines. During pregnancy, Th2-type immunity predominates, i.e., inflammation is downregulated so that the fetus is supported and not rejected. At term, there is reversal of the Th1/Th2 cytokine ratio, with IFN-Y-induced activation of cell-mediated immunity. This is a possible mechanism for overcoming trophoblast-induced immunosuppression, and inducing "rejection" (i.e., parturition) of the fetus.

The results to date are preliminary. Data from only 6 of the 8 plates run have been processed at this time; processing of the remaining 2 plates is ongoing and will be completed shortly. Thus, some of the patterns we have seen so far may become stronger or less strong with the complete n/group. In addition, further processing is needed using data reduction techniques such as PCA, constrained PCA, network analysis, preparation of hierarchically clustered heatmaps etc., to see the bigger picture of cytokine functional networks. Moreover, we need to relate our results to other biological measures (Miranda, Sarkar) and to demographic, psychosocial, alcohol/drug use, SES, health-related activities, and other measures that have been obtained on the women. We will be visiting Dr. Chambers' laboratory to work with her, Jordan Schafer, and Matt Rolland on February 22-23, 2016. As well, we have been interacting with Dr. Miranda to begin to link our data with his. In this context, we make our conclusions below with caution at this time.

To date we have seen three major patterns of cytokine levels, and results are presented accordingly:

a) Alcohol-consuming moms, *regardless of whether the child is affected* (groups A and B) differ from moms who had low to no ethanol consumption (group C) – an effect of ethanol consumption *per se.*

Placental growth factor (PGF) is produced by the placenta, and is critical in vasculogenesis (formation of the vascular network in the embryo), and angiogenesis (remodeling and expansion of this network). Levels of PGF increased more steeply in controls than in women who consumed alcohol (Fig 1). While the are no differences in VEGF or VEGF receptor (VEGFR1) among groups, the VEGFR1/PGF ratio, a marker of risk for pre-eclampsia or miscarriage, is lower in women who drink than controls. Lower levels of VEGFR1 and PGF have been associated with miscarriage.

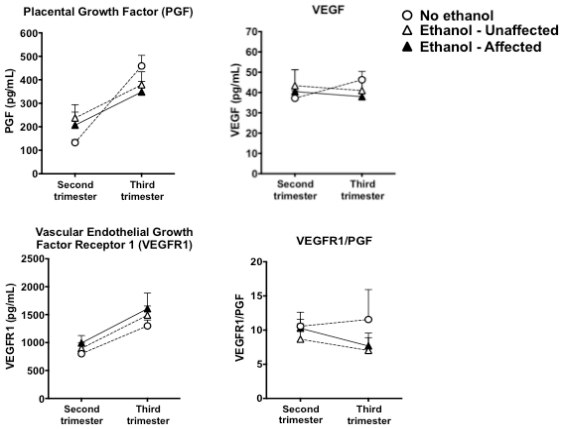


Fig 1. Four angiogenic proteins, all members of the vascular endothelial growth factor (VEGF) family, were altered.

In addition, we found higher levels of granulocyte-macrophage colony stimulating factor (GM-CSF, Fig 2) and IL-2 (Fig 3) in women who drink compared to controls. GM-CSF plays a key role in embryo implantation and subsequent development. It is possible that higher GM-CSF levels in women who drink represent a compensatory mechanism to maintain the pregnancy in these women. By contrast, IL-2 is a pro-inflammatory cytokine, which is known to increase at the end of the third trimester and can be measured in serum during labor. Thus, we can speculate that the lower levels/lack of increase

in women who drink is a maladaptive response that may relate to delayed labor/delivery. On the other hand, higher levels of the anti-inflammatory cytokines, IL-10 and IL-13, during the second trimester possibly represent an adaptive response in alcohol-consuming women to protect the fetus.

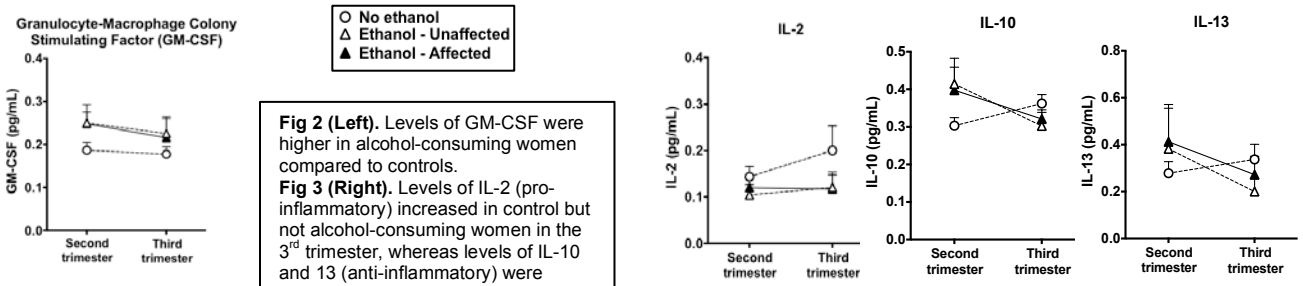


Fig 2 (Left). Levels of GM-CSF were higher in alcohol-consuming women compared to controls.
Fig 3 (Right). Levels of IL-2 (pro-inflammatory) increased in control but not alcohol-consuming women in the 3rd trimester, whereas levels of IL-10 and 13 (anti-inflammatory) were higher in alcohol-consuming women vs controls in the 2nd trimester.

b) Alcohol-consuming moms with *affected* children (group A) differ from both alcohol-consuming moms with unaffected children and moms with low-no ethanol consumption (groups B and C).

Levels of three pro-inflammatory cytokines (Fig 4, Left panel, IFN-γ, IP-10, IL-17A), and one anti-inflammatory cytokine (Fig 4, Right panel, IL-5) were elevated in women in group A compared to those in groups B and C. We can't yet fully interpret these findings. However,

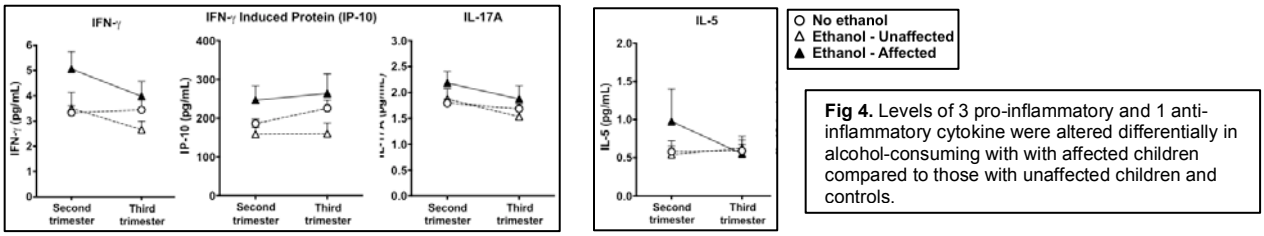


Fig 4. Levels of 3 pro-inflammatory and 1 anti-inflammatory cytokine were altered differentially in alcohol-consuming with with affected children compared to those with unaffected children and controls.

findings from the prenatal stress literature have shown that increased pro-inflammatory Th1 cytokines in the mother (maternal-fetal interface) are linked to higher risk of allergy and other immune (particularly auto-immune) disturbances in the fetus. Thus, it is possible that higher levels of Th1 cytokines in the mother will link to changes in the child's immune response. Elevations of anti-inflammatory cytokines could arise in response to the increased pro-inflammatory cytokines. However, the two do not balance each other one to one, and, as noted above, higher levels of anti-inflammatory cytokines during the second trimester possibly represent an adaptive response in alcohol-consuming women to protect the fetus.

c) Alcohol-consuming women with *unaffected* children (B) differ from those with affected children and those with low-no alcohol consumption (groups A and C).

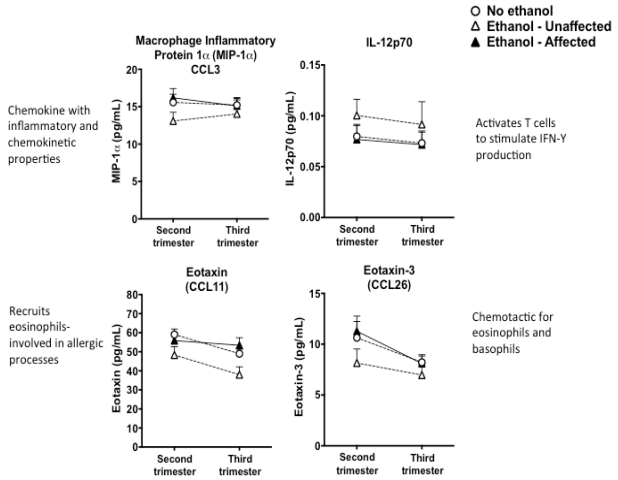


Fig 5. Four chemokines were differentially altered in women who consumed alcohol but who had unaffected children compared to women with affected children and controls

Four chemokines (Fig 5) were differentially altered in alcohol-consuming women with unaffected children compared to women in the other two groups. As seen in Fig 5, these chemokines are primarily involved in inflammatory processes and recruitment of immune cells. However, further work is needed to understand the functional importance of these findings.

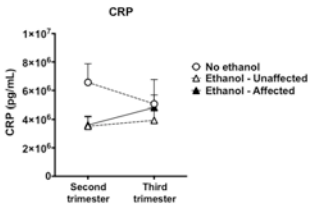


Fig 6. CRP levels are lower in alcohol-consuming women compared to controls at the 2nd trimester.

Finally, our preliminary analysis of CRP (Fig 6) shows a marked difference between alcohol-consuming women and controls during the second but not the third trimester. Further analysis will examine ratios between CRP and cortisol to gain further insight into the consequences of this outcome.

3. For this reporting period (April 1, 2015 - present), 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?

This project has provided unique opportunities for professional development of the key people from my lab who are involved in and leading the work – Dr. Charlis Raineki, a senior Research Associate, and Tamara Bodnar, a senior PhD student who will continue as a Postdoctoral Fellow on this project. We are primarily a basic neuroscience research lab, utilizing animal models of prenatal alcohol exposure to examine the effects of alcohol exposure *in utero* on brain, biological, and behavioral development over time. While I have developed a number of clinical collaborations over the years, this is the first collaboration to focus on FASD, and the first where students/Postdocs/Research Associates are working with me to play a key role in developing and leading collaborative clinical research. Indeed, Charlis and Tamara were key in developing the research proposal for this Developmental Project, and continue to play a leading role in managing and carrying out the project. This project has given Charlis and Tamara the opportunity to extend their knowledge and talents from “the bench to the bedside,” and have hands-on experience in a clinical research project. It has also been fantastic to work with Dr. Chambers and her team to understand how her cohorts have been characterized and the extensive information already obtained on the women and children in this study, to develop criteria for choosing the samples to run, and to begin an extensive analysis of the data. As noted, we will be visiting her lab on Feb 22-23 to work with her, Jordan Schafer and Matt Rolland on advanced data analysis. We have also had the chance to interact with Rajesh Miranda and will be interacting further in trying to link our data sets to each other and to all of the other outcomes measures on the women and their children.

The University of British Columbia does not have formal IDPs for our students. Plans for PhD students are developed collaboratively with me and then presented to their Supervisory Committees, comprised of faculty from various departments who are experts in their field of research. Plans with senior personnel, such as Research Associates, are also developed collaboratively with me and colleagues in relevant areas of research.

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

Nothing to report at this time.

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

This project is directly linked to Dr. Tina Chambers’ project, “Early identification of affected children and risk factors for FASD in Ukraine.” We are analyzing plasma and saliva samples collected from pregnant women and children in cohorts recruited in Ukraine. To our knowledge,

this is the first human study in the FASD field to use extensive and complementary endocrine and immune measures to identify the immune/neuroimmune phenotype of women drinking alcohol during pregnancy and the immune/neuroimmune profile of children with FASD in relation to that seen in the control (no alcohol exposure) condition. The significance of our proposal derives from the potential to link immune and endocrine phenotypes and correlate these with physical, neurobehavioral, social, environmental, cognitive, health, and other outcomes, which together will extend ongoing CIFASD research. The combination of salivary and plasma measures of the developing immune system in the children is especially novel, as it may identify minimally invasive biomeasures feasible for use in clinical populations. If we can elucidate novel FASD biomarkers through this approach, this will support the possibility of developing novel and targeted immune-based interventions.

7. What do you **plan to do for the next reporting period** (March 1, 2016 - April 1, 2017) to accomplish your project's specific aims?

Please note, our first year of funding on this project ends on May 31. Briefly, we will complete the Year 1 goals by May 31, and carry out activities toward our Year 2 goals during the next reporting period.

From now until May 31, we will complete our analysis of cytokines with extensive high-level data analysis. Once the final two cytokine plates are processed, we will do some initial analyses, including constrained principal component analysis (CPCA) and hierarchically clustered heatmaps, to begin to form a picture of cytokine networks that may be differentially activated in the different groups of women. We have already seen some interesting patterns, as reported above, but more extensive analysis is needed to link the various cytokines to each other and gain insight into overall patterns of response. We will bring these analyses to San Diego on February 22-23, and these will provide the basis for further more global analyses, linking our measures to all of the other measures - social, psychological, demographic, health-related, biological, etc - obtained on these women. In addition, we will work with the bioinformatics specialists at UCSD to evaluate the impact of nutritional supplements on our cytokine, cortisol and CRP measures.

As noted, our Year 1 goals were to analyze blood from the pregnant women to assay cytokines, cortisol and CRP. We are currently completing the analysis of cytokines and CRP, as noted. Cortisol assays should be completed in March. In addition, based on the literature, and in consultation with Dr. Chambers, we have decided to add one additional measure - plasma levels of corticotropic releasing hormone (CRH). Accumulating evidence suggests that fetal exposure to placental CRH (pCRH) can program developmental trajectories and play a key role in mediating the effects of maternal stress on infant and child development. During the second and third trimester, the human placenta produces high levels of CRH (concentrations rise over 1000-fold during pregnancy), most of which is secreted into the maternal bloodstream. pCRH stimulates secretion of maternal ACTH and cortisol, with substantial increases (2-4-fold) in maternal serum cortisol during the third trimester. The normative exponential increase of cortisol and pCRH over the latter part of pregnancy plays a fundamental role in the organization of the fetal nervous system, influences the maturation of the fetal HPA axis and other systems, modifies birth phenotype (timing of onset of spontaneous labor and delivery), and regulates maternal adaption during pregnancy. Thus, pCRH appears to play a role in coordinating effects of endocrine, immune/inflammatory, and vascular processes on fetal developmental outcomes.

Importantly, the human placenta integrates numerous sources of maternal stress signals and responds with a dose-dependent release of pCRH. In turn, the placenta/fetal unit responds to this pCRH increase by itself synthesizing and releasing CRH from the hypothalamus (positive feedback) and activating a cascade of hormonal, physiological and metabolic consequences.

For the mother, pCRH may mediate the association between early life support/adversity and her postpartum state (eg., postpartum depression). For the fetus, depending on timing during gestation and severity of maternal stress, there may be a significant impact on the developmental trajectory, with effects on growth, stress system regulation, immune function/ inflammation, and metabolic function, as well as motor, cognitive and emotional function. Moreover, the rate of change or rise of CRH production over the course of gestation has been shown to reflect maternal adversity and thus provides another important marker of maternal state. Moreover, as the rise in CRH is critical for onset of parturition, a steeper rise may be related to pre-term birth and poorer child outcome. As we have blood from both the second and third trimesters of pregnancy, we will be able to determine whether the rate of change of CRH differs in women consuming alcohol compared to women in the control group, and how this relates to maternal conditions and fetal outcomes.

In summary, measurement of pCRH during pregnancy can provide insight into stress and adversity experienced by the mother as well as the impact of maternal stress on the fetus. In the present study, we propose that pCRH could provide a novel biomarker for maternal stress and may provide a link to adverse child outcomes in a wide variety of domains.

During the next reporting period, June 1, 2016 – May 31, 2017, we will carry out activities toward our Year 2 goals – analysis of endocrine and immune measures in the children, as well as assessment of the impact of nutritional supplements on both mothers and children.

Our proposal is to utilize blood and saliva samples currently being collected from children in the CIFASD Ukrainian cohort (ranging in age from 2 – 4 years), including children in each of the alcohol exposed/unexposed conditions, with or without nutritional intervention, who are coming back to the clinic for follow up assessment, to examine both salivary and plasma markers of immune and endocrine function. When blood samples are available, levels of 40 cytokines will be measured, as we did for the maternal blood samples, using the same Human Biomarker 40-Plex Kit from MesoScale Discovery, including pro- and anti-inflammatory cytokines and chemokines, angiogenesis and vascular injury markers, and CRP. The MSD system will allow us to maximize our use of the scarce and highly valuable CIFASD blood samples, measuring the highest number of analytes in the smallest possible sample volume. Cortisol will also be assayed in these samples. As with the pregnant women, our goal is to identify an immune signature in this cohort of children with FASD. Moreover, and in line with the ongoing CIFASD investigation, we will investigate whether nutritional supplementation can modulate the expected proinflammatory profile. One limitation is that women and children may come from different cohorts and thus may not be perfectly matched. Nevertheless, the analyses should provide valuable information of endocrine-immune function of the children, enable us to assess the effects of different levels of prenatal alcohol exposure, and allow us to correlate endocrine-immune alterations with multiple other outcome measures in these children.

In addition, when available, saliva samples will be assayed to measure cortisol and CRP, allowing us to calculate a cortisol/CRP ratio, a combined measure of endocrine/immune function, parallel to what we will do with plasma measures. The cortisol/CRP ratio provides insight into cortisol release in the context of an inflammatory state (e.g., low cortisol expression in the context of heightened inflammation), thus providing further insight into the nature of immune and/or endocrine system dysregulation. Importantly, this will also allow for comparisons to be made between plasma and salivary CRP levels. Should levels be found to correlate, conclusions can be drawn regarding the inflammatory status of children from which plasma could not be obtained.

As immune deficits observed in children with FASD are hypothesized to arise due to combined effects of alcohol exposure, maternal endocrine dysregulation, and increases in maternal

cytokine levels, the maternal cortisol/CRP ratio, together with the cytokine profile, and measures obtained on the children should increase our understanding of health status of the mothers and health outcomes of the children.

The first batch of 59 blood samples from children in Ukraine has now arrived in the US, so we are optimistic that we will be able to obtain blood samples from cohorts of children for our analyses during Year 2. As an alternative approach, if for some reason we cannot obtain sufficient blood and saliva from children for analysis, we are exploring, with Dr. Chambers, other possible sources for samples from children with FASD. One possibility is to use blood spots from children with FASD and matched controls from the Biobank in the state of California, which Dr. Chambers heads. Blood spots from the Biobank can now be requested, at a small cost, to use in research. While the Biobank has no records on maternal alcohol intake, blood spots from children whom Dr. Ken Jones has assessed can be identified and requested for analysis. Additionally, we may be able to obtain blood spots from children in other CIFASD cohorts (e.g., Atlanta). Thus, one way or another, we believe that we will have blood samples from children with FASD and children with no alcohol exposure to analyze during Year 2 of our project.

Over the next few months we plan to write a manuscript based on the data from the maternal blood samples. As we approach the end of Year 2 we hope to be able to write a manuscript based on data from children.

8. CIFASD Phase III ends in May of 2017. **What specific aims could you see your project addressing in the upcoming competitive renewal?**

We are currently partway through our first year of funding and are thus relatively new to CIFASD. However, we have a number of ideas on how we might move forward with the work begun, and continue to work with Dr. Chambers and her team to extend our findings in the context of her studies.

Analysis of matched blood and saliva samples from mothers and infants.

New cohorts of pregnant women are being recruited currently in Ukraine. As we could be involved relatively early in this new cohort, we should be able to obtain blood from the women at 2 times during pregnancy and from their infants at birth. Having matched samples from mothers and children will extend our current studies where we may not be able to match mother-child pairs directly (or at least will likely not obtain very many mother-child pairs), and thus provide novel insight into how alcohol-induced alterations in the mother/intrauterine environment during pregnancy might directly translate into physiological, neurobiological, neurobehavioral alterations in the children, and how these alterations relate to multiple other outcome measures. Further, based on our current data, we should be able to significantly streamline the analysis of cytokines and other markers to focus on those that show the most promise.

Measurement of placental 11β-HSD1, 11β-HSD2, and glucocorticoid receptors (GR).

Cortisol levels are 5-10-fold higher in the maternal circulation than in the fetal circulation, a gradient thought to be maintained by high placental 11-β hydroxysteroid dehydrogenase (11β-HSD2) levels. 11β-HSD2 catalyzes the conversion of maternal cortisol to cortisone before the active steroid can reach the umbilical vein, and thus functions as a physiological feto-placental glucocorticoid barrier, protecting the fetus from overexposure to maternal glucocorticoids. Conversely, 11β-HSD1 catalyzes conversion of inactive steroid to cortisol, thus regenerating and amplifying glucocorticoid action. These enzymes contribute to the intracellular ‘‘gating” of glucocorticoid action and thus play a critical role in glucocorticoid signaling in the placenta and intrauterine glucocorticoid exposure. Moreover, evidence suggests that these enzymes play a significant role in developmental programming, and may do so via epigenetic mechanisms. In the placenta, the *HSD11B2* gene, which encodes the 11β-HSD2 enzyme, is regulated by DNA

methylation, and appears to be vulnerable to stressors from the maternal environment. For example, birth weight, a measure of intrauterine growth, and infant quality of movement, an early neurobehavioral outcome, were shown to be inversely associated with the extent of methylation of the *HSD11B2* gene promoter. Thus, regulation of fetal stress via *HSD11B* appears to be a major factor in fetal growth and development, with DNA methylation potentially playing a key role in this process.

Additional studies have examined the joint contribution of the placental glucocorticoid receptor (*NR3C1*) and *HSD11B2* in infant neurobehavior, based on the possibility that examining patterns across the cortisol regulation pathway would provide an enhanced representation of HPA dysregulation compared to that obtained when examining these factors separately. Once again, epigenetic mechanisms appear to play a role in the separate and combined influence of these factors and could provide important information on the molecular basis for fetal origins of mental health.

Data from our animal model studies demonstrate alterations in GR signaling in the placenta of females consuming alcohol during pregnancy. Interestingly, we also saw increased 11β-HSD2 expression in placentae of alcohol-consuming females, which may be a compensatory response to protect the fetus from increased glucocortiocid hormone levels.

Placenta samples are being collected in cohorts currently being recruited at the Khmelnytsky Perinatal Center, and will possibly be collected at another hospital as well. The protocol for collection is currently being refined. Access to placental tissue for analysis will provide an exciting opportunity for elucidation of novel markers of alcohol's effect on the fetus. Investigation of placental expression of 11β-HSD1, 11β-HSD2, and GR can provide novel insights into the intrauterine environment, and in particular, fetal exposure to glucocorticoid hormones, which are known to play a role in fetal programming, poor neurodevelopmental outcomes, and vulnerability to adult onset diseases or disorders. We have the expertise to examine placental expression of 11β-HSD1, 11β-HSD2, and GR in our laboratory. Of particular interested, we could possibly partner with other CIFASD investigators who have expressed interest in analyzing placental tissue, including Rajesh Miranda, Scott Parnell, and Jeff Wozniak. This would greatly extend and enhance this aspect of the proposed research.

The possibility of assessing alterations in epigenetic marks reflective of alcohol exposure in this same placental tissue is also of great interest. In discussions with Dr. Chambers, it was suggested that Dr. Kelly Frazer in the Division of Genome Information Sciences at UCSD, who did the epigenetic analyses for the CIFASD samples, would be a great collaborator for this aspect of the project. Importantly, we will connect with other CIFASD researchers who are interested in this area to examine possible epigenetic mechanisms that may mediate the actions of alcohol on the placenta and the fetus, and which may serve as novel biomarkers for prenatal alcohol exposure.

Analysis of inflammatory markers in breast milk linked to analysis of the infant gut microbiome.

A recent publication using a mouse model describes a "lactocrine" pathway, through which reduced maternal levels of several cytokines and chemokines resulted in reduced milk levels of these same cytokines and chemokines, with positive consequences for hippocampal cell proliferation, leading, in turn, to enhanced learning and memory. Our findings during Year 1 of this project suggest that the opposite may be occurring in our population, i.e., alcohol-induced increases in maternal plasma levels of most of the same cytokines and chemokines reported in the animal model study. Thus we suggest the possibility that enhanced levels of cytokines might be transmitted to nursing offspring through the milk, and might adversely impact neurobiological development of the offspring. We propose to measure inflammatory and stress markers, such

as immunoglobulins, cortisol and cytokines, in the milk of women consuming alcohol and their abstinent counterparts. We would work directly with Dr. Chambers to gain access to new birth cohorts currently being recruited. The new cohorts of pregnant women currently being recruited in Ukraine are one possible source of samples for this work. As well, we would hope to partner with Dr. Ludmila Bakhireva at the New Mexico Alcohol Research Center, and with CIFASD researchers at UCSD and in Atlanta who are recruiting new birth cohorts. If we can work together with researchers throughout the CIFASD network, this would provide a more diverse population, with differential patterns of drinking, genetic backgrounds, SES, etc, and greatly strengthen the work.

In addition, we propose to assess the gut microbiome of the children of these women. The microbiome is the collection of microbes or microorganisms that inhabit our bodies, creating a sort of “mini-ecosystem”. These microorganisms exist in unique, complementary blends, and can be found on the skin, genitals, mouths, eyes, and intestines. For the purposes of this proposal we will focus on the gut microbiome. The digestive tract of a newborn is rapidly colonized with microorganisms from the mother and the surrounding environment, and is markedly influenced by breastfeeding. The presence and relative abundance of key groups of bacteria has a strong impact on health and survival, including aiding in digestion and the production of certain vitamins such as vitamins B and K and influencing immune function. Moreover, the gut microbes produce chemicals, called metabolites, which can be detected in fecal samples. Increasing evidence indicates that the gut microbiome influences our risk for diseases such as obesity, auto-immune diseases like diabetes, rheumatoid arthritis, muscular dystrophy, multiple sclerosis, fibromyalgia, and perhaps some cancers, as well as mental health problems such as anxiety and depression. The microbiome may also play a role in the development of autism.

In the context of the known gut-brain axis, it is possible that *in utero* alcohol exposure may have an impact on bacterial populations that colonize the gut and/or impact fetal gut development, with subsequent impacts on brain development. For example, chronic alcohol consumption is associated with an increased abundance of proinflammatory gut microbes and a decrease in normal commensal bacterial. However, it remains to be determined whether alterations in the balance of bacteria in the maternal system have an influence on the gut microbiome of the offspring. It has also been shown that changes in glucocorticoid levels around the time of parturition affect gut maturation and closure. We hypothesize that *in utero* alcohol exposure may delay and/or impair gut permeability such that material (gut microbes, metabolites produced by gut microbes) could more easily leave the gut, triggering an immune response, and stimulating cytokines production both peripherally and centrally. Altered communication between gut and brain could have numerous downstream effects on multiple aspects of health and development. Alcohol-induced changes in the gut microbiome is one possible mechanism underlying the pro-inflammatory bias (i.e., increased plasma and brain levels of pro-inflammatory cytokines) seen in our animal model following prenatal alcohol exposure and could underlie inflammatory changes that we expect may be observed in children with FASD.

Measurement of the gut microbiome in a cohort of children with FASD and matched controls could thus provide tremendous insight into the vulnerability of these children for diseases or disorders later in life. Moreover, understanding changes in the gut microbiome that occur following prenatal alcohol exposure could provide novel targets for treatment of children with FASD that might attenuate at least some of the adverse effects of prenatal alcohol exposure. This line of research would link beautifully with both the current CIFASD focus on nutrition and growth, as well as our current Developmental Project and the proposed collaborative extension of this work on immune function/inflammation in children with FASD for the CIFASD renewal.

In collaboration with Dr. Chambers and Dr. Bakhireva, we hope to gain access to fecal samples collected from newborns and children in the newly recruited Ukraine cohorts and in cohorts being recruited at the New Mexico Alcohol Research Center. In addition, it may be possible to assess children recruited by investigators in the CIFASD consortium over the past 11 years in various US sites including LA, San Diego and Atlanta. In discussions with Dr. Chambers, it was suggested that we could work with Dr. Rob Knight at UCSD, a world-renowned expert in microbiome research, on this aspect of the project. We know that Dr. Knight and Dr. Chambers are part of the group going forward to submit a grant for the ECHO call for proposals, so he is already part of that research team along with Dr. Chambers.

Intervention in children with FASD: A novel immune-based approach.

Data from our animal model work on neuroimmune function demonstrates a pro-inflammatory bias in offspring that were prenatally exposed to alcohol. The current preliminary results of our Developmental Project indicate significant differential cytokine patterns in the three groups examined - women who drank alcohol during pregnancy and had children who were affected, women who drank and had unaffected children, and women who drank low levels or no alcohol during pregnancy. Together, these findings suggest the possibility that immune-based interventions could provide a novel treatment approach for both women who drink alcohol and their affected children.

In view of the complexity of initiating a treatment in humans, we believe it is essential to pilot one or more immune-based interventions in our animal model of prenatal alcohol exposure prior to planning a trial in children/adolescents with FASD. A logical initial starting point is to pilot the efficacy of minocycline in attenuating inflammation induced by alcohol exposure *in utero*. Minocycline is a broad-spectrum antibiotic and putative microglial inhibitor, shown to decrease inflammatory cytokine production, with promising clinical trials results. We will treat developing animals during the adolescent period, a sensitive time in brain development. Minocycline will be administered for 1-2 weeks (to be piloted) via drinking water to half the animals in each of the prenatal alcohol, pair-fed and *ad libitum*-fed control conditions, with the remaining animas receiving water alone. Minocycline levels will be monitored during administration. Both the immediate (at the end of adolescence) and long-term (in adulthood) effects on neuroendocrine/neuroimmune function of the offspring will be examined. Offspring will undergo testing to assess anxiety-/depressive-like behavior in the open field, elevated plus maze, and light-dark box. Males and females from each prenatal group/treatment condition will then receive a lipopolysaccharide (LPS) or saline challenge, blood samples collected at key times post-injection, and animals then terminated for collection of brains. We will measure levels of key pro- and anti-inflammatory cytokines and chemokines (as determined from our previous studies) and CRP, as well as microglial activational status within key brain areas. Plasma levels of corticosterone, CRP and cytokines will also be measured (again, targeted as per our current results). This comprehensive study should provide critical information on the efficacy of minocycline in attenuating the pro-inflammatory profile observed following prenatal alcohol exposure.

Building on the findings of this study, we would hope to initiate trials to treat children with FASD with minocycline. This would require extensive collaboration with investigators in CIFASD who have ongoing studies with/recruitment of children with FASD, and who have expertise in clinical work and clinical trials. We would aim to treat adolescents rather than younger children, in order to avoid staining of the enamel as a complication of minocycline treatment in young children whose permanent teeth are still forming. While mounting a clinical trial is a formidable undertaking, the advantage of our proposal is that it will be based on extensive preclinical data (from previous studies to determine safety and efficacy and our proposed animal study specifically targeting prenatal alcohol exposure), and proposes to use minocycline, a drug

known to be safe and well tolerated, and currently being administered in clinical trials on children with autism.

9. What do you believe the **hot areas of FASD research** to be?

Neuroimmune function/inflammation

Child health outcomes

Biomarkers of maternal alcohol consumption, maternal stress/adversity, adverse child outcomes

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

None.

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

No publications have been submitted yet.

12. **Poster Abstracts and Presentations**

An abstract based on our data has been submitted to RSA 2016 for a poster presentation:

Bodnar T., Raineki C., Wertelecki W., Yevtushok L., Zymak-Zakutnya N., Weinberg J., Chambers C.D., the CIFASD. Alcohol Consumption During Pregnancy Alters Maternal Immune parameters: Implications For The Offspring. Alcohol Clin Exp Res, in press (to be presented at the Research Society on Alcoholism, New Orleans, LA, June 2016).

Principal Investigator(s): Tatiana Foroud
Institution(s): Indiana University
CIFASD Project Title: Genomewide SNP Analysis
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.

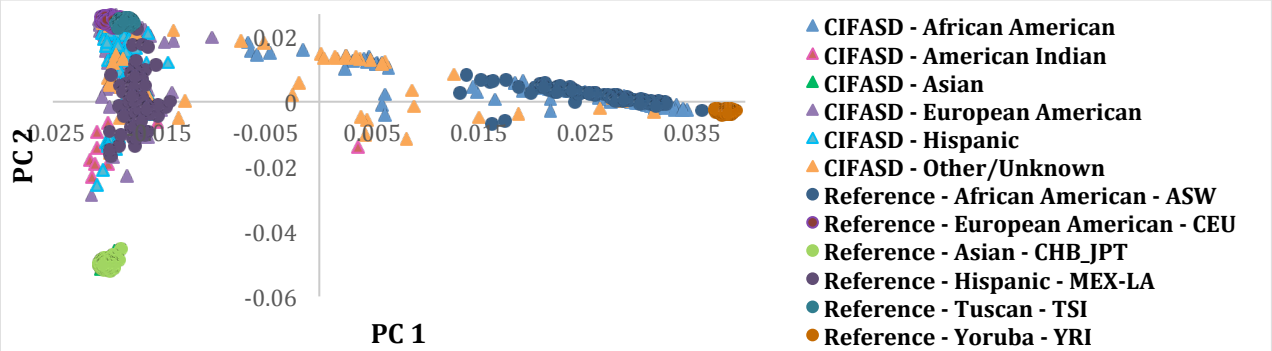
Samples from 308 individuals were genotyped using the new MEGA array which includes over 2 million single nucleotide polymorphisms (SNPs) and was optimized for samples of diverse ancestry. Data from these samples were combined with genotypes previously genotyped on an Illumina array for 236 CIFASD participants. All genotypic data underwent imputation to expand the number of SNPs available for analysis. The merged data set after quality control contained 2,328,149 genotyped and imputed SNPs in 544 individuals. The enlarged sample now includes 264 Caucasian (48.5%), 195 African American (36%) and other (primarily Hispanic and Asian, 15.5%) individuals. There are 243 individuals with heavy exposure to alcohol and 306 with minimal or no exposure. A little more than half the sample is male (55.7%).

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.

Combining the SNP genotypes generated in the CIFASD sample (triangles) with the SNP genotypes from publicly available data (circles), we have generated principal components that reflect the ancestry of the samples (see Figure 1). Shown in this figure are principal components 1 and 2. Principal component 1 (PC1) along the X axis reflects European-African ancestry (from left to right) and separates the African American samples with varied European and African admixture from those of European ancestry. The Y axis (PC2) reflects European-Asian ancestry from top to bottom. In the CIFASD sample, this axis distinguishes those of Native American and Hispanic ancestry from other Caucasian (European ancestry) populations.

These DNA-based principal components can now be included as covariates in analyses and provide the ability to utilize quantitative estimates of ancestry rather than self-reported ancestry. We have utilized the principal components in preliminary genetic analyses and are now uploading these variables into the Central Repository for use by others in CIFASD. They can also be used as covariates in non-genetic analyses, including analyses of the facial images, neurobehavioral measures and brain images.

Figure 1: Principal Components Generated from the SNP Genotyping Array Data



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

We have confirmed that the same SNP in *PDGFRA* which was associated in the original GWAS sample is associated in the larger combined sample with a newly constructed measure of palpebral fissure length developed from the 3D facial imaging data. Similarly the same SNP providing evidence of association in *BMPR1B* is associated in the combined sample with the same philtrum phenotype based on principal components from the 3D data. This same SNP also demonstrates association with a composite phenotype of key executive function phenotypes.

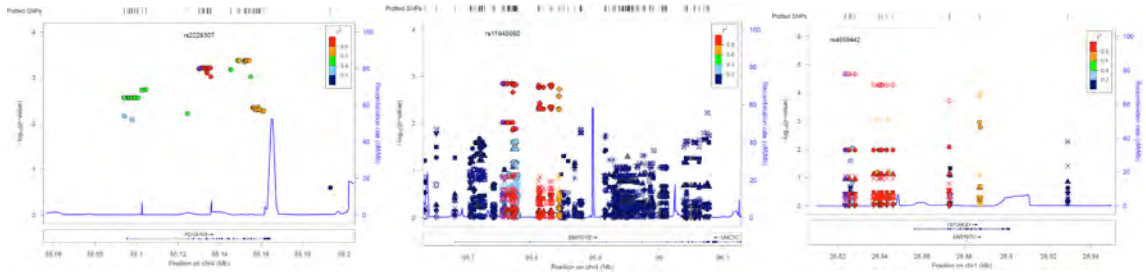


Figure 2: Left to right: *PDGFRA*, *BMPR1B* and *RPS6KA1*. Each symbol represents one SNP, high y-axis values indicate significant association. Warm colors denote high genetic correlation with the purple SNP, which is the SNP of interest.

The association of SNPs in the Src kinase gene FYN did not replicate in the combined sample. We believe this is due to the very low minor allele frequency observed for those SNPs in the original sample. However, examination of other Src genes yielded interesting results for *RPS6KA1* and *ANK3*. SNPs in these genes are associated with several facial phenotypes as well as executive function and corpus callosum. These findings are under further review, to determine the veracity of the association.

3. For this reporting period (April 1, 2015 - present), 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?

This continues to be an opportunity for training and professional development for Leah Wetherill, who is a doctoral student in the Department of Psychology. Her dissertation involves genetic underpinnings of prenatal alcohol exposure.

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

RSA Symposium 2016: THE FACE, BRAIN, AND BEHAVIOR IN FASD: CONNECTING THE DOTS IN CIFASD; MODELING THE ROLE OF GENETICS IN THE PHENOTYPIC VARIATION OF FAS (Foroud, Tatiana)

6. Describe your project's **interrelation** with aims of the CIFASD consortium and other CIFASD 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).

7. What do you **plan to do for the next reporting period** (March 1, 2016 - April 1, 2017) to accomplish your project's specific aims?

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 will continue to interact with the Basic Science projects to explore the effects of prenatal alcohol exposure on specific phenotypes of interest, and how those effects are mediated by genotype for the specific genes put forward by the Basic Science investigators.

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.

8. CIFASD Phase III ends in May of 2017. **What specific aims could you see your project addressing in the upcoming competitive renewal?**

I am concerned that the sample size for analyses remains small and we are struggling to obtain robust, reproducible results.

9. What do you believe the **hot areas of FASD research** to be?

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

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

12. **Poster Abstracts and Presentations**

RSA Symposium 2016: THE FACE, BRAIN, AND BEHAVIOR IN FASD: CONNECTING THE DOTS IN CIFASD; MODELING THE ROLE OF GENETICS IN THE PHENOTYPIC VARIATION OF FAS (Foroud, Tatiana)

Principal Investigator(s): Johann K. Eberhart
Institution(s): University of Texas at Austin
CIFASD Project Title: Genetic Screens in Zebrafish to Identify Gene-Ethanol Interactions
Grant Number: U24 AA014811 (Administrative Core of the CIFASD)

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

Aim 1: Identify genetic loci mediating susceptibility to FASD

Aim 2: Elucidate the neural and behavioral correlates of gene-ethanol interactions

Aim 3: Characterize pathways that protect against ethanol-induced teratogenesis.

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

Accomplishments related to Aim 1: Aim 1 combines forward genetics and targeted analyses of the Wnt/PCP pathway to explore gene-ethanol interactions. We previously identified a forward genetic mutant and genetically mapped the lesion to the *lrp13b* locus. We have now generated a CRISPR/Cas9-inudced lesion in *lrp13b* and phenocopied the original mutant. Lrp13b is poorly characterized with no analyses suggesting it plays a role in ethanol teratogenesis, at least to our knowledge. Thus, we have strong evidence that *lrp13b* is a novel ethanol-sensitive mutant. We have generated riboprobe against lrp13b to begin analyzing its expression. The Lrp family of gene products mediates many different signaling pathways (interestingly, this includes the Wnt/PCP pathway), often through regulation of endocytosis. We are currently generating transgenic zebrafish, with fluorescently labeled components of the endocytic pathway to determine if ethanol disrupts this process.

Our analyses of ethanol-treated *vangl2* mutants continue, in which ethanol induces cyclopia in mutants and a portion of heterozygotes. We have demonstrated that ethanol causes a convergent/extension defect in embryos that is exacerbated by loss of *vangl2*. We have performed RNA-seq analyses in wild-type embryos to identify ethanol-induced alterations to the transcriptome that may cause the phenotype that we observe.

Accomplishments related to Aim 2: Here we proposed to characterize gene-ethanol interactions in a set of transgenic lines that label various neural populations and recently established behavioral paradigms in zebrafish. Using the neural progenitor labeling *elavl3:EGFP* transgenic line, we are currently determining the sensitivity of neural progenitor cells to varying concentrations of ethanol. We are examining this same line to define a possible peak time of neurogenesis to allow us to test if this time window is particularly sensitive to perturbation by ethanol. We are generating a set of transgenic zebrafish lines that we hope will be very useful for FASD research. First, using an E-SARE promoter region, from the *Arc* locus, we have constructed an *E-SARE:dsEGFP* transgenic construct. We are currently testing if this construct is activated in zebrafish, as it is in mouse, by neural activity. Second, the expression patterns of neurochemicals regulating social and reward behaviors are highly conserved, even from human to fish. We have generated riboprobe for all three of these receptors and are currently using PCR-based and CRISPR/Cas9 knock in approaches to generate transgenic zebrafish labeling cells expressing *oxytocin*, *dopamine d1a* and *vasopressin 1a*.

We have initiated social behavioral analyses using ethanol-treated *pdgfra* heterozygous versus wild-type fish. We are currently testing the shoaling behavior of these fish using a paradigm of a 2-hour ethanol administration at 16 and 24 hpf. We are working to develop learning paradigms that would be amenable to high throughput analyses. We have evidence that zebrafish rapidly develop a place preference following short, repeated visualization of a computer generated shoal. We are working to establish a similar protocol based on zebrafish avoiding a stimulus of a computer-generated predator.

Accomplishments related to Aim 3: Here we proposed analyses of how the Hsp90 and mTOR pathways interact to regulate sensitivity to ethanol. We are currently generating genetic resources for this Aim. Notably we have generated CRISPR gRNA to target *rraga*, a critical component of the mTORC1 pathway.

3. For this reporting period (April 1, 2015 - present), 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? Nothing to report.

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

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

We have interacted with Dr. Scott Parnell in comparing genetic results and writing a comprehensive review on gene-ethanol interactions. We have interacted with Dr. Foroud to explore evidence for conservation in human of the gene-ethanol interactions that we identify in zebrafish.

7. What do you **plan to do for the next reporting period** (March 1, 2016 - April 1, 2017) to accomplish your project's specific aims?

We will obtain mutagenized sperm to initiate our large-scale forward genetic screen for Aim 1. Within the year we should have our first ethanol-sensitive mutants and we be begin to perform genetic and phenotypic characterizations on these mutants. In Aim 2, we will have finished a set of behavioral analyses in at least 3 sets of ethanol-sensitive mutants, *pdgfra*, *vangl2* and *kif3b*. We will begin characterizing the neural circuitry in those mutants with defective behavior, using our E-SARE transgenics or *elavl3:Campari* transgenics, in which active neurons are labeled following UV light exposure. In Aim 3, we will have generated a set of mutant and transgenic resources within the Hsp90 and mTOR pathways to test for their modulation of ethanol sensitivity.

8. CIFASD Phase III ends in May of 2017. **What specific aims could you see your project addressing in the upcoming competitive renewal?**

We envision a collaborative proposal with Dr. Scott Parnell that could address the following questions: 1) Identification of an evolutionary conserved ethanol-sensitive network. Using techniques such as RNA-seq across mouse and zebrafish, we could map conserved ethanol-induced transcriptomic alterations. 2) High throughput analysis to identify gene-ethanol interactions. New CRISPR/Cas-9 techniques allow for mutant analyses in the F1 generation. We would use this technique for both global and tissue specific knock down of gene function to rapidly identify gene-ethanol interactions. 3) Multifactorial analyses of FASD risk.

9. What do you believe the **hot areas of FASD research** to be?

Biomarkers, genetics, prevention/suppression of FASD.

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

McCarthy N, Bertrand, JY and Eberhart JK (accepted pending minor revision) An Fgf-Shh signaling hierarchy regulates early specification of the zebrafish skull. Developmental Biology.

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

Eberhart JK and Parnell SE. The Genetics of Fetal Alcohol Spectrum Disorders (FASD), ACER, submitted Feb 2016.

Lovely CB, Fernandes Y and Eberhart JK. Fishing for FASD: zebrafish as a model for ethanol teratogenicity, Zebrafish, submitted Feb 2016

Loverly CB, Swartz ME, McCarthy N, Norrie JL and Eberhart JK. Bmp signaling mediates endoderm pouch morphogenesis by regulating Fgf signaling in zebrafish, Development, revision to be submitted Feb 2016.

Lovely CB, Fernandes Y and Eberhart JK. Gene-environment interactions and development, SDB wire, to be submitted March 2016.

12. **Poster Abstracts and Presentations**

McCarthy N (grad student), Lovely CB, Swartz ME and Eberhart JK. A zebrafish forward genetic screen identifies enhancers of ethanol teratogenesis, Presented as a poster at the annual RSA meeting. San Antonio, TX. June 2015.

Sidik A (grad student) and Eberhart JK. Attenuation of the planar cell polarity pathway may cause susceptibility to FASD, Presented as a poster at the annual RSA meeting. San Antonio, TX. June 2015.

Lovley CB (post doc). Zebrafish genetic screens identify ethanol susceptibility loci. Oral presentation, FASDSG, San Antoinio, June 2015.

Lovely CB (post doc). Zebrafish genetic screens identify ethanol susceptibility loci. Platform presentation, Teratology Society meeting, Montreal, QC. June 2015.

-Winner of 2015 Wilson Presentation award

Lovely CB (post doc). Zebrafish genetic screens identify ethanol susceptibility loci. Platform presentation, TRSA, College Station, TX, June 2015.

Brock C (undergrad). Sequencing and determining the ethanol sensitivity of the *b1101* mutation in zebrafish. Oral presentation, Texas Academy of Sciences, San Antonio, TX, March 2015.

Eberhart JK. Getting ahead, using genetic screens in zebrafish to model craniofacial disease variation. Invited seminar, University of Kansas. Lawrence, KS. April 2015.

Eberhart JK. Using genetic screens in zebrafish to identify ethanol-sensitive loci. Plenary session presentation, 6th International Conference on FASD. Vancouver, BC. March 2015.

Principal Investigator(s): Dipak K. Sarkar
Institution(s): Rutgers, The State University of New Jersey
CIFASD 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 alcohol feeding in rodents during the early postnatal period, equivalent to third trimester of human pregnancy, induces *Per2* gene hypermethylation and suppresses *Per2* mRNA levels in the hypothalamus, lowers *Pomc* mRNA expression in the hypothalamus, and elevates corticosterone level in plasma 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 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 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 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 epigenetic marks 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 and POMC-derived peptide beta-endorphin levels were determined in fetal alcohol exposed children. Salivary samples were collected at two CIFASD sites (University of Minnesota and Children Hospital at Los Angles) and used for measurement of these proteins by ELISA. We found that the salivary cortisol level in control and FASD children maintains a circadian profile; higher in the morning and lower in the late afternoon. The cortisol level in the FASD children were moderately elevated, but not significantly, in the morning but markedly and significantly elevated in the late afternoon, as compared to those in control. The beta-endorphin level also show a circadian pattern in saliva. However, beta-endorphin levels in the FASD children were significantly, lower in the morning and in the late afternoon, as compared to those in control. These data identify an association between POMC and PER2 DNA methylation and increased stress hormone levels in saliva.

We also used maternal blood DNA samples from the Ukrainian patient cohort provided by Dr. Tina Chambers at UCSD. Using these samples, we determined whether POMC and PER2 gene methylation changes could be detected in mothers during pregnancy who gave birth to FAS or

FASD children. We found the levels of POMC and PER2 gene methylation were significantly elevated in the blood samples of mother who abused alcohol during pregnancy and gave birth to a FAS child or an unaffected child as compared to those in non-drinker mother. These data suggest that prolonged alcohol use during pregnancy increases DNA methylation of POMC and PER2 genes that persist in children with FAS or FASD.

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 persist throughout childhood. This is a significant development to the field, since they identify putative biomarkers for fetal alcohol exposed subjects with the risk of vulnerability for developing neuroendocrine diseases.

3. For this reporting period (April 1, 2015 - present), 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. Omkaram Gangisetty received postdoctoral training.

Miss Shaima Zabar received predoctoral training.

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

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

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

7. What do you **plan to do for the next reporting period** (March 1, 2016 - April 1, 2017) to accomplish your project's specific aims?

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.

8. CIFASD Phase III ends in May of 2017. **What specific aims could you see your project addressing in the upcoming competitive renewal?**

My major objective will be to determine specific epigenome and/or histone isoforms for FAS and for FASD. I will investigate this question by employing CIFASD resources and addressing the following aims:

- Conduct differential DNA methylation analysis in biological fluids of FAS and FASD children to identify FAS and FASD specific methylation changes
- Determine if FASD and FASD specific methylation changes can be detected in biological fluids of mothers who abused alcohol and produced FASD or produced non-FASD children
- Conduct differential histone analysis to identify any histone isoforms specific for FAS or FASD
- Study if the FAS or FASD-related histone isoforms can be detected in biological fluids of mothers who abused alcohol and produced FASD and non-FASD offspring

9. What do you believe the **hot areas of FASD research** to be?

Detection of the biological marker(s) of FAS and/or FASD using genomic or proteomic techniques.

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

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

I am currently putting together a manuscript containing these and other relevant data for submission to a journal.

12. **Poster Abstracts and Presentations**

I have submitted an abstract entitled, “Identification of alcohol-induced epigenetic marks in maternal-child pairs” in the symposium proposed by Dr. Michael Charness entitled “ Early identification of gestational alcohol exposure and fetal alcohol spectrum disorders in the CIFASD cohort” for 2016 ISBRA meeting in Berlin, Germany.

Principal Investigator(s): Alison Noble
Institution(s): University of Oxford
CIFASD Project Title: Prenatal Face-Brain Analysis in FASD
Grant Number: U24 AA014811 (Administrative Core of the CIFASD)

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

To define protocols

- for image capture of the fetal face that improves the existing coverage of the facial mid-line
- to enable neural structures such as the corpus callosum to be captured and subsequently segmented from the 3D ultrasound image

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

Collaboration has been established with Dr. Aris Papageorghiou of the Nuffield Department of Obstetrics and Gynaecology whose clinical and research interests include obstetric ultrasound, normal and abnormal fetal growth, pre-eclampsia and fetal diagnosis and treatment. Dr. Papageorghiou has facilitated the acquisition of new 3D ultrasound volumes to add to those provided previously by the PASS network.

Because of Peter Hammond's move from UCL to Oxford on August 1st 2015, he was unable to assist with this development project in June and July. In addition, detailed analysis had to wait until October 2016 for the arrival of a doctoral candidate from China, Ruobing Huang, who has chosen to focus her research on the application of machine learning to 3D ultrasound image analysis with a particular emphasis on fetal alcohol syndrome and related differential diagnoses. Ms. Huang previously completed an MSc in the Engineering Department at Oxford including an individual project within the Noble research lab. So far she has undertaken familiarization with the FASD literature and has attended several talks by and meetings with Peter Hammond to understand the dense surface modeling approach to face shape analysis and its use within the CIFASD consortium.

The work on prenatal 3D ultrasound data undertaken by a previous doctoral student in Dr. Noble's lab, Dr. Tom Rackham, was based on 20 or so images provided by the PASS network. That work was based on slices of each image derived from animations delineating a sagittal traverse through the volume which proved necessary because the commercial proprietary format precluded access to the underlying raw sonographic image data. Ms. Huang has had similar problems in extracting the raw data despite trying several commercial ultrasound viewing platforms. While these difficulties are being overcome, she has been working closely with a postdoctoral researcher, Dr. Ana Namburete, who completed doctoral studies in the Noble lab in 2015 on the application of machine learning techniques to predict gestational age and neurodevelopmental maturation from 3D fetal ultrasound images of the brain. Ms Huang has been able to apply her newly acquired technical ultrasound analysis skills to segment the corpus callosum of a fetus from the 3D ultrasound images.

The previous ultrasound study combining ultrasound segmentation and DSM based shape analysis established a statistically significant correlation between mid-line facial profile and prenatal alcohol exposure. More recent analysis has shown that the perinasal region can discriminate postnatally between FAS and 3 differential diagnoses (Cornelia de Lange, Williams and 22q11 deletion syndromes) almost as well as the full face. This suggests that any protocol adopted for future prenatal analysis of FASD related features should ensure adequate coverage of both the mid-line profile and the nose.

3. For this reporting period (April 1, 2015 - present), 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?

Initial focus for a doctoral student recruited in October 2015.

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

Nothing to report.

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

Close collaboration has been maintained with Peter Hammond and Mike Suttie to ensure compatibility of prenatal and postnatal face oriented data to support longitudinal studies of individual facial growth and development.

7. What do you **plan to do for the next reporting period** (March 1, 2016 - April 1, 2017) to accomplish your project's specific aims?

Current funding does not support this development project.

8. CIFASD Phase III ends in May of 2017. **What specific aims do you see your project addressing in the upcoming competitive renewal?**

Prenatal face and brain analysis to support earlier diagnosis of *in utero* alcohol exposure.

9. What do you believe the **hot areas of FASD research** to be?

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

None.

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

Ready for submission awaiting PASS network approval:
T Rackham, M Suttie, L Wetherill, T Foroud, ...R Meyer, H Nolan, H Odendaal, A Noble. Optimizing 3D ultrasonography of the fetal face to detect the effects of prenatal alcohol exposure.

12. **Poster Abstracts and Presentations**

None.

Principal Investigator(s): Rajesh C. Miranda
Institution(s): Texas A&M Health Science Center
CIFASD Project Title: Circulating microRNA Biomarkers of Fetal Alcohol Exposure
Grant Number: U24 AA014811 (Administrative Core of the CIFASD)

1. What are the **major goals** of the project? The major goals of this administrative supplement were to purchase enough qRT-PCR microRNA arrays to complete assessment of 136 samples of plasma from a Ukraine Cohort of pregnant women (part of U01 AA014835, Chambers, PI).

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

Major Activities and Objectives. We were able to complete all of the 136 samples by November 2015. A more complete account of progress is provided in the progress report for U01 AA014835. Briefly, we assessed plasma miRNA profiles in mid and late pregnancy in 68 women. This includes 22 women with known alcohol consumption with affected infants (Group-A), 23 women with known alcohol consumption with apparently un-affected infants (Group-B), and 23 control women (Group-C).

The objective, to complete miRNA assessments, extract data and share all data with the Chamber's research group, was accomplished by December 2015. Current objectives are to analyze the data and create predictive statistical models.

Significant results. Our preliminary analysis indicates that there are statistically significant differences in miRNA expression between groups, when adjusting for multiple comparisons. A random forest analysis of Groups A and C, with the inclusion of clinical variables, can achieve an Overall Misclassification Rate: 11.96%. This means that ~88% of samples in group A and C can be correctly assigned to their respective groups. In contrast, the random forest approach has a misclassification rate of 33.7% for the Group A vs. B comparison and a misclassification rate of 14.3% for a Group B vs. C comparison. We also examined the extent to which plasma samples obtained from Group B women could be classified as either more like Group A or C. Our analysis indicate that by the end of pregnancy, ~80% of the samples in group 'B' were classified as more like Group 'A' rather than 'C'. These data suggest that alcohol-exposed mothers with apparently non-affected infants may be preferentially classified with alcohol-exposed/affected infant group, on the basis of plasma miRNA profiles during pregnancy.

3. For this reporting period (April 1, 2015 - present), 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?

Nothing to report.

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

Nothing to report.

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

This project was conducted in collaboration with Dr. Tina Chambers, U01 AA014835. It is directly related to the aims of this project in assessing the biomarker potential of maternal miRNAs for infant outcomes in alcohol-exposed women. Other projects within the consortium are also identifying ways to diagnose fetal alcohol exposed children earlier and more accurately. We think that there may be future synergy between our plasma miRNA project and for example, projects U01AA014809 and U01AA014834. It would be useful to determine whether the miRNA profiles have discriminatory value in other populations and whether these profiles could predict other neurobehavioral, brain structure and face outcomes.

7. What do you **plan to do for the next reporting period** (March 1, 2016 - April 1, 2017) to accomplish your project's specific aims? In collaboration with the Chamber's research group, we will finish the analysis of this data, and intend to publish the findings.

8. CIFASD Phase III ends in May of 2017. **What specific aims could you see your project addressing in the upcoming competitive renewal?**

There are several important issues that could be explored in a competitive renewal.

Replicability and cross-cultural validity: It would be important to determine the extent to which an miRNA based predictive model could predict infant outcomes in a new patient sample, from the Ukraine and from other FASD cohorts in the US or elsewhere. It might also be important to determine whether the changes in maternal miRNA content are replicated in the infant. If that is the case, infant blood (cord blood perhaps) could be used as an alternative tissue source for biomarker analysis, should maternal samples be unavailable.

Predictive value: In this current project, maternal plasma miRNA profiles are being related to a small number of early infant outcomes. Does a maternal profile have predictive value for later infant and child development? Can knowing something about the mother enable us to predict brain structural and neurobehavioral outcomes, scholastic performance?

Specificity: Are the changes in plasma miRNA profiles specific to maternal alcohol exposure or are these changes generalized markers for maternal-fetal distress? By screening the published literature, we will be able to determine the extent to which neural tube defects, pre-eclampsia or other pregnancy associated complications result in overlapping patterns of maternal miRNA alterations. In the current study, smoking is likely to be an important contributory factor to maternal miRNA changes. However, it would also be important to determine if, for example, maternal opioid exposure could result in similar changes in maternal plasma miRNA content.

Therapeutic relevance: Increasing evidence indicates that secreted miRNAs may be act as endocrine factors. There is a possibility that we may be able to deliver compensatory doses of miRNAs to the mother and that this may improve the trajectory of fetal development, so that the effects of earlier adversity are decreased. This approach would require going back to preclinical models of fetal alcohol exposure and testing the therapeutic efficacy of miRNA delivery.

9. What do you believe the **hot areas of FASD research** to be?

I believe that early and quantitative diagnosis of FASD through the entire spectrum will be an important and hot area. CIFASD may be best positioned to develop, through meta-analysis of epigenetic (including miRNA), structural and functional data, good predictive models that will facilitate early, perhaps prenatal, interventions.

An equally important and 'hot' area will be medications development. Can we use our understanding of compromised molecular processes to develop novel therapeutic approaches? I believe that secreted miRNAs are well suited to be manipulated as programming factors for modulating maternal immune system, placental growth and other factors that may facilitate fetal growth after an adverse maternal experience.

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

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

Once we have a firm grasp of the outcomes of our data analysis, Dr. Chambers and I plan to submit a CIFASD concept proposal, and plan to write up this data for publication in the next few months.

12. **Poster Abstracts and Presentations** None.

Site Investigator(s): Jeffrey R. Wozniak
Institution(s): University of Minnesota
CIFASD Project Title: Supplement to the Administrative Core of the CIFASD
Grant Number: U24 AA014811 (Administrative Core of the CIFASD)

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

Prior to joining CIFASD, our site provided CIFASD with access to 52 research participants with FASD. All of these participants were seen for dysmorphology exams by Ken Jones, had DNA collected, and had 3D facial photographs taken by Tatiana Foroud's team. With the administrative supplement, we collected neurobehavioral data and neuroimaging data on those extra participants – once we formally joined CIFASD.

A second goal that evolved for the use of the administrative supplemental funds was to facilitate our site's ongoing collection of 3D facial images, DNA, salivary hormones for Dipak Sarkar's developmental project, and in-person dysmorphology exams (all tasks that have no site budgets of their own).

During the period covered by this progress report, we utilized the remaining funds allocated for April 1, 2015 – May 31, 2015 (a two-month period that ended with the end of our no-cost extension) to support the above research activities (via covering extra subject payments and travel expenses, etc.).

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

For the period covered here, administrative supplement funds were used to support the second goal: to facilitate our site's ongoing collection of 3D facial images, DNA, salivary hormones, and in-person dysmorphology exams (which have no site budgets of their own).

3. For this reporting period (April 1, 2015- May 31, 2015: the brief period of no-cost extension that is covered by this reporting period), 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?

Nothing to report.

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

Nothing to report.

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

The aims for the use of these administrative supplemental funds were directly tied to the Neuroimaging project and the Neurobehavior project initially (allowing for collection of additional data for participants who were enrolled prior to our formal participation in CIFASD.) Since then, our use of the administrative supplemental funds has been directly tied to the 3D facial imaging project and the Dysmorphology core – supporting those activities directly. Lastly, we have also used these funds (for subject payments, materials, freezer storage, etc.) to support collection of saliva for Dipak Sarkar's developmental project.

7. What do you **plan to do for the next reporting period** (March 1, 2016 - April 1, 2017) to accomplish the goals?

If additional administrative supplemental funds are available in 2016, we will continue to use those funds to support our site's collection of 3D facial images, DNA, salivary hormones (Dipak Sarkar's developmental project), and in-person dysmorphology exams (which have no site budgets of their own).

8. CIFASD Phase III ends in May of 2017. **What specific aims could you see your project addressing in the upcoming competitive renewal?**

NA – this progress report covers supplemental funds from the Administrative Core only.

9. What do you believe the **hot areas of FASD research** to be?

Intervention research, especially neurocognitive efforts and biological interventions – possibly nutritional in nature. Another hot area will be epigenetics and/or the continued search for biomarkers. Although perhaps not within reach yet, abbreviated/automated diagnostic tools would be a huge move forward in our field.

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

Nothing to report.

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

Nothing to report.

12. **Poster Abstracts and Presentations**

Nothing to report tied to these administrative funds.

Administrative Core Equipment Supplement Update:

Principal Investigator(s): Julie Kable
Institution(s): Emory University
CIFASD Project Title: GoFar Therapy for FASD Children: Prefrontal Blood Oxygenation Changes Using fNIR
Grant Number: U24 AA014811 (Administrative Core of the CIFASD)

We are actively collecting data now and it is my intention to submit a grant this summer using the fNIR as an outcome measure for our GoFar intervention. I have not attempted to compare group data yet and limited myself to analyzing the results to determine if the protocol is working and it seems to be. I am using two tasks. The first is the Tasks of Executive Control, which is a commercially available computerized task of executive functioning skills. The task incorporates a 0-back, 1-back, and 2-back condition (this one is only for kids > 7) which are administered with and without an inhibitory (no-go) component. This allows me to look at changes in blood oxygenation levels as a function of the inhibitory condition (yes or no) and as a function of the task difficulty relative to working memory. We started using this task in our med management clinic with FASD patients to assess the impact of various medications and I decided to use this task as part of the fNIR protocol as a result of its usefulness in the clinic. The other advantage is that I get standardized scores from the software regarding the participant's performance. The test was developed by the same group who did the BRIEF which has been useful in differentiating FASD neurobehavioral problems. The second task is the FETCH game that was described in the development grant proposal that was submitted to CIFASD. This game has the participant compete with a computer dog to grab a bone as quickly as possible when it flashes on the screen. The task was originally developed for preschoolers and I was concerned about its use with older children and teenagers but from the initial pool of subjects that we have ran, the game appears to be equally appealing to all of the participants in CIFASD that we have run. I have been keeping track of behavioral observations and comments while they are performing the task, which are quite amusing. My only concern with the game is whether or not the keyboard on the laptop will survive running too many participants as many have resorted to hitting the space bar hard thinking this will help them beat the dog to the bone. I may have to budget for replacement equipment in the next grant. This task has 7 blocks with 4 being winning trials and 3 being losing trials. The trials alternate W-L-W-L-W-L-W. In the win condition, the participant is able to win 4 of 5 bones offered and in the losing trials only 1 of 5 and the speed of the participant's response has no real outcome on whether or not they win or not. Affective ratings by participants indicate that all perceived the losing blocks as more aversive than the winning blocks and blood oxygenation levels vary as a function of the winning/losing condition. The latter can be detected at the individual subject level.

At the CIFASD meeting, I would be happy to do a brief presentation and by that time I would probably have enough to do group comparisons (FASD, Behavioral Contrast, and Typical Controls). We have run 12 participants to date but anticipate doing 8-10 a month now that we have human subjects approval and have worked out the procedures and protocols.

In addition to the group comparison's with the CIFASD subjects, I also plan to assess the relationship between the amplitude of the blood oxygenation levels in the various conditions and the neurobehavioral outcomes collected as part of Sarah's protocol (i.e., CBCL, BRIEF, subtests from the CANTAB, NEPSY) but I would anticipate this would not be ready until next Fall sometime when we have had a chance to collect 80-100 subjects.

Principal Investigator(s): William K. Barnett
Institution(s): IU, UITS, Advanced Biomedical IT Core
CIFASD Project Title: Informatics Core for the Collaborative Initiative on Fetal Alcohol Spectrum Disorders
Grant Number: 5 U24 AA014818-12

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

Specific Aim 1: Continued cyberinfrastructure support.

Aim 1.1: The Informatics Core will continue to provide data management and integration and will lead the ongoing development of the CIFASD data dictionary, architectures for managing CIFASD data, and tools for data quality improvement and exploratory data analysis. Aim 1.2: It will continue to provide reporting support to the Administrative Core to track consortium progress.

Specific Aim 2: Collection of additional data sets.

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.

Specific Aim 3: Support of affiliated projects.

Aim 3.1: The Informatics Core will create a permissions system so that data from affiliated studies may be securely shared. Aim 3.2: The Informatics Core will work with affiliated studies to allow their data to be integrated into the central repository or otherwise compared with CIFASD project data, if affiliate study data are not structured or described in the same fashion as CIFASD studies.

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

The following specific aims were addressed during this period:

Specific Aim 1: Continued cyberinfrastructure support.

Aim 1.1: The Informatics Core will continue to provide data management and integration and will lead the ongoing development of the CIFASD data dictionaries, 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.

The Informatics focused on the following priorities: supporting the aggregation of additional data from CIFASD clinical research projects into the CIFASD Central Repository (CR), improving the functionality of data collection standards and tools, and assisting CIFASD investigators with data integration needs for cross-study analyses.

As of February 12, 2016, the CR had aggregated data on a total of 22,198 subjects (an increase of 4,113 from the past half year). The informatics Core:

- Identified and corrected erroneous Global ID for various datasets based on users' request.
- Updated Neurobehaviour III data dictionary, the Access input tool and central repository with new value ranges for NEPSY II variable.
- Standardized Screener, Ultrasound, 2nd Interview (Follow-up/Outcome), and Eating Habits Data dictionaries prior to final data upload to the central repository.

- Implemented “Data in Spreadsheet View” option on the Switchboard for Screener, EEAC, and Eating Habits to assist users in rapid data access and download of individual datasets to the comma-separated values format.
- Included new discrepancies reports to various datasets to improve quality of the data uploaded to the CR.
- Improved the XML export functionality for the Screener, Ultrasound, 2nd Interview (Follow-up/Outcome), and Eating Habits Access input tools.
- Modified input forms for EEAC, Screener, Ultrasound, 2nd Interview (Follow-up/Outcome), and Eating Habits to provide total number of records per form in the form’s footer.
- Improved the Demographics Access input tool:
    - Created an additional automated functionality to update records with totals prior to exporting data in XML format.
    - Added a discrepancy report for records with uncalculated totals for Hollingshead and Puberty Questionnaires.
- Adjusted the Demographics Static query output algorithm to allow Static data to be retuned independently from the date it was attained.
- Re-developed the presentation of Demographics Alcohol and Medical records to permit multiple Alcohol and Medical records on one line but not in one field as previously depicted.
- Altered the algorithm for retrieving Dysmorphology records on Neurobehavior and Cross Query pages based on the rank of administrator.
- Notably improved query performance by implementing materialized views for Mother-Child related datasets (such as EEAC, Screener, Ultrasound, and 2nd Interview), as well as Dysmorphology, and Neurobehaviour Static data.
- Submitted CIFASD monthly data reports to the Administrative Core documenting data uploaded into the Central Repository. The report was modified to include newly added datasets, such as Screener, Ultrasound, Follow-up/Outcome and Eating Habits.
- Developed a dynamic report for “Central Repository Update Statistics” that depicts the most recent data inserts, updates and deletions of all essential datasets per day.
- Rebuilt all Phase II and Phase III DemGroupClass and Completeness reports in cifasd.uits.iu.edu to eliminate dependency on limited security provided by current Oracle Application Express version.
- Modified the Central Repository 'Reports' page to link directly to the “Phase III Tallies%” page.
- Established a procedure to consistently update links to the public data sharing data dictionaries and Access input tools on www.cifasd.org site.
- Created secure storage on the box.iu.edu document repository to streamline exchange of the Access input tools between CIFASD ‘power’ users on the principal of the least privilege.

Specific Aim 2: Collection of additional data sets.

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 (CR). 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.

During the past year, the Informatics Core completed revising the Cross Query Tool that allows investigators to do self-directed integrative queries of CIFASD data in the CR. We also assessed if these tools are suitable, in terms of security and patient privacy protection, for use

by external investigators, and implemented a user access and data delivery process using REDCap for logging security and data presentations. In particular, we

- Added new datasets to the Central Repository:
    - Screener (~10,000 records imported: Khmelnytsky - 9305, Rivne - 9016, Moscow - 413)
    - Ultrasound (imported: Khmelnytsky - 539, Rivne - 893)
    - 2nd Interview (imported 1265 records combined for Outcome and Follow-up datasets)
    - Eating habits (imported: Khmelnytsky-184, Rivne - 152)
- Included new variables, updated existing variable names, cleaned up invalid variables in the Brain Imaging data dictionary and central repository based on the FreeSurfer output.
- In collaboration with several consortium sites and with assistance of Ben Deweese, Leah Wetherill, Jeff Wozniak, Sarah Mattson, and Jordan Schafer the Informatics Core led the process of clarification of the cross query requirements and essential functionality that culminated in development of a dynamic reporting tool (AKA Cross Query) that streamlined data retrieval from multiple datasets to promote greater data usage by CIFASD researchers.
- Implemented various data filters on Neurobehavior and Cross Query pages that allowed restriction of the data output and simplification of data download.
- Created data upload, duplicate checking, and download functionality for the 2nd Interview (Follow-up/Outcome), Ultrasound, Screener, and Eating Habits datasets.
- In addition to providing access to all datasets currently available in CR including Infant and Preschool Neurobehaviour, EEAC, 2nd Interview (Follow-up/Outcome), Ultrasound, Screener, and Eating Habits, the Informatics Core
    1. added new filters including
        - different Date of Administration dependencies between datasets (Closest Date, Earliest Date, Most Recent Date) and
        - an option to restrict downloads of identifiers and produce de-identified record set.
        - the ability to output complete subject records that has data in all tables.
    2. provided interactive reporting capabilities to dynamically restrict the data output before downloading. The options include:
        - search by any column in which user can employ semicolon (;) to search for multiple values in the field.
        - select rows (multi-row select) that could be accomplished by pressing the SHIFT and CTRL /CDM keys on the keyboard to add / remove rows from the selection.
        - download data in two formats: 1) copy records to the clipboard and paste them to Excel or other programs in tab-delimited format; or 2) save records in CSV format. In both cases, there are options to save just a subset or a complete record set.
        - show/hide columns.

- Implemented ‘Save Report’ option on Cross Query page to assist researchers in efficient retrieval of the previously defined data queries.
- Created Mother-Child connection for the datasets without child related Global IDs (such as EEAC, Screener, Ultrasound, and 2nd Interview.

Specific Aim 3: Support of affiliated projects.

Aim 3.1: The Informatics Core will create a permissions system so that data from affiliated studies may be securely shared. Aim 3.2: The Informatics Core will work with affiliated studies

to allow their data to be integrated into the central repository or otherwise compared with CIFASD project data, if affiliate study data are not structured or described in the same fashion as CIFASD studies.

During the past year, the Informatics Core

- Led the Data Access Committee and created a Data Access Policy, Data Use Agreement, and online Data Request Form for external collaborators who want to pursue relevant research questions with CIFASD data
- Finalized identification of Phase I data by projects and sites in preparation for providing data access to the external researchers.
- Designed and implemented a section on the public cifasd website (cifasd.org) that provides external researchers with granular access to CIFASD Phase I data based on their signed agreement with CIFASD consortium.

3. For this reporting period (April 1, 2015 - present), 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?

To stay abreast of technology and help with in resolving various CIFASD issues, Helen Yezerets undertook training in Advanced Database Security and Database Design.

Advanced Database Security course provided a strong foundation in database security and auditing. It covered topics on implementation of administration policies for users' profiles and password policies, privileges and roles, Virtual Private Databases using roles and application context, and auditing. Advanced Database Design course concentrated on the best practices of sound database design from gathering essential requirements for the information system to adjusting data models for the top database performance.

Informatics Core underwent HIPAA Privacy and Security Training V2.

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

The Informatics Core led the Data Access Committee and created a Data Access Policy, Data Use Agreement, and online Data Request Form for external collaborators who want to pursue relevant research questions with CIFASD data. We finalized identification of Phase I data by projects and sites in preparation for providing data access to the external researchers. We designed and implemented a section on the public CIFASD website (cifasd.org) that provides external researchers with granular access to CIFASD Phase I data based on their signed agreement with CIFASD consortium, and worked with the Data Access Committee to launch this section publicly.

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

The Informatics Core is essential infrastructure for CIFASD as a whole. The structure of a separate Informatics Core has facilitated the collaborative processes that have enabled the consortium's researchers to come to consensus on data definition and measurement issues that are essential to the broader goals of the CIFASD as a whole. We believe that this can be a model for FASD research programs in the future. The work of the Informatics Core has led to the creation of data dictionaries that will ensure that the common data collected by the consortium are usable and understandable indefinitely, the creation of a Central Repository of data from clinical research projects, and the suite of computer tools we have created that ensure the data are easily accessible.

7. What do you **plan to do for the next reporting period** (March 1, 2016 - April 1, 2017) to accomplish your project's specific aims?

The Informatics Core will continue to work with the entire consortium to provide new data input, management, and output services, understand the ongoing needs for software tools and their development, provide better tools for data analysis, and ensure data quality. As needs change and the scope of data coming into the Central Repository expands, there will be a continued need for Informatics Core staff to assist researchers with data uploads and data cleansing in the central repository. There also will be new needs for tool modification as data upload needs, methodologies, or contributing sites change. As projects create new data types, such as genomic data, and affiliate projects create data integration requirements, the Informatics Core stands ready to assist in those endeavors in terms of data normalization, management, and reuse for cross-cutting and other studies.

The focus of the consortium continues to shift from the emphasis on collecting to analyzing and comparing data among affiliated projects. The Informatics Core will continue to be a facilitator for CIFASD clinical projects, support existing data submissions, and develop technical solutions for cross-study and/or cross-modality data analysis. In addition to modifying existing input and upload tools, we will shift focus to improving query and analytical capabilities for both hypothesis-driven analysis and quality assurance.

We will explore new formats that can be used to export data (such as SPSS).

The Informatics Core will also continue to work to support data quality improvements by fine-tuning existing reports and providing new analytics, summaries, and visualizations of CIFASD data.

Finally, we will commit our resources for provisioning of CIFASD data for external requests, consultations and training. The Informatics Core will manage the new process for external investigators to gain access to CIFASD data and to oversee the data sharing process. The Informatics Core will also provision data to external investigators and provide long term archiving of derived data sets and other informatics services as per the CIFASD Data Use Agreement.

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

- Continue providing maintenance, changes, and enhancements to various software tools.
- Provide custom datasets, as requested.
- Develop a user manual to clarify the best methods of interaction with CIFASD data via External User Access website.
- Prepare publications and presentations to raise awareness of CIFASD as a data resource for FAS research and lower barriers to access by external investigators.
- Assist researchers outside of CIFASD to access Phase I data through public cifasd website.
- Identify Phase II data and prepare it for External User Access.
- Report records with calculated totals for Hollingshead and Puberty data on the Demographics discrepancy report.
- Depict presence or absence of demographic collection points to signify completeness of the Demographics data.
- Accommodate second time tested Phase III Neuro records to restrict their visibility only in connection with Brain Imaging data (MRI) if requested at the same time.
- Improve presentation of Cross Query report by freezing a first (header) row of the report.

- Develop functionality to track subjects with a 3D image and DNA and include these datasets on the Cross Query page.
- Implement dynamic range data filtering on Neurobehaviour Query page report in accordance with Cross Query report.
- Develop data analytics via reports, summaries and data visualizations, as requested.

8. CIFASD Phase III ends in May of 2017. **What specific aims do you see your project addressing in the upcoming competitive renewal?**

The initial Informatics priority for CIFASD had been the creation of tools to enable data collection. At the start of 2015, these data input tools were largely created, though work refining them continues. The emphasis of efforts then became continuing to collaborate with the sites to create central repositories for collected data, developing upload/download capabilities for remaining datasets based on changing consortium needs, and ensuring data integrity by identifying erroneous records and dependencies. In addition, we streamlined access to the collected data for analysis for internal researchers through dynamic cross-query report, and created access for external researchers to the completed data phase.

The emphasis of the Informatics Core's work will continue to support the creation of data input tools for new data sets and types, and collecting data provided by CIFASD and, in future, external collaborators. In 2017 and beyond, the primary efforts of the Informatics Core will shift to collecting new data types (such as genomic data), implementing more effective methods for extraction and analysis of the data that has been collected, and facilitating data access and data dissemination among non-CIFASD community of researchers.

9. What do you believe the **hot areas of FASD research** to be?

We anticipate that the upcoming renewal period will be signified by data provisioning not only for internal CIFASD users, but also for external users. In addition, as Phase II period is getting closer to its final stage, we will update External User Access website with Phase II data. We are excited about the possibility that incorporation of new data types, such as genomic data, will enable in better understanding FAS. Finally, we believe that cross data analytics and data visualization will play significant role in better understanding the relationship among data and in pursuing the scientific goals of CIFASD.

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

Nothing to report.

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

Yezerets, H. (Y.), Arenson, A., Barnett, W.K., CIFASD. Role-based access control (RBAC) policy for HIPAA Aligned Research Data, Manuscript in preparation. CIFASD concept proposal form submitted in November 2015.

12. **Poster Abstracts and Presentations**

Nothing to report.

Principal Investigator(s): Kenneth Lyons Jones
Institution(s): University of California, San Diego
CIFASD Project Title: Dysmorphology Core
Grant Number: 5 U24 AA014815-12

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

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

Aim #2: To develop a training DVD that could be used to teach physicians and other health care professionals with little or no experience in diagnosis of FASD to correctly identify the characteristic structural features of FAS through a physical examination and to successfully diagnose of 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: Between April 1, 2015 and February 12, 2016, I performed 91 physicals examinations in five clinical sites throughout the world. These included 30 physical exams in Atlanta, Georgia, 0 physical examinations in Los Angeles, CA, 36 physical examinations in Minneapolis, Minnesota, 16 physical examinations in San Diego, CA and 9 physical examinations in Ukraine.

To accomplish this goal the previously established CIFASD physical examination protocol and classification system is used to perform and/or validate physical examinations of all infants and children who were participants in the CIFASD renewal project and who were not previously examined by the Core examination team.

In addition, the previously established CIFASD examination training protocol provides on-going training and re-training of local pediatricians/neonatologists/geneticists who are performing preliminary examination at some CIFAS sites. In some sites, over the last year I have worked face-to-face with the local examiners to maintain quality of their physical examinations.

Aim #2: I have developed 15 minute DVDs of children with the fetal alcohol syndrome. The purpose of these DVDs is to teach pediatricians and other health care professionals how to perform a physical examination that will allow them to identify the characteristic physical features of FAS and how to diagnose it. This careful physical examination focuses on minor anomalies that are characteristic of this disorder. In addition, a DVD has been recorded which focuses on the neurobehavioral features of Neurobehavioral Disorder Associated with Prenatal Alcohol Exposure (ND-PAE).

To determine the effectiveness of this tool, a comparison was made using a 10 question FAS Quiz, between five pediatric residents who completed their training by using the DVD exclusively and those who completed hands-on live training done by me. The comparison is based on their ability to correctly identify features of FASD as well as their ability to make a diagnosis of FASD. There was no difference between the two groups.

In addition, the San Diego Chapter of the American Academy of Pediatrics has agreed to provide a copy of the DVD to all 500 members of the local AAP for their review and comments. They have asked and we have agreed to transcribe it by streaming as opposed to sending them the DVD. This will be streamed to them in mid-March and we are hoping to get feedback from them by mid-April.

Aim #3: There are two phases that we have used to provide long distance consultation.

Phase I: Pilot Consultations: The remote physicians have the capability to film the physical exam and securely transmit them via the internet to me in San Diego for review and thereafter to schedule a consultation. For this consultation, the remote clinicians look at the participant documents and video at their site and I look at the same information on the secure repository at my site. In July 2015 this phase was successfully completed at the University of Minnesota where we are collaborating with Dr. Jeffrey Wozniak and was outlined in our last report.

Phase II: Real-Time Consultations: This phase gives physicians and healthcare providers the capability to perform the clinical examination and consult with me during a live session at the remote site in order to make the diagnosis of FAS or successfully rule out this disorder. Real-time consultations give clinicians in remote sites the benefit of interactive consultation with the patient present. The goal is to provide an effective clinician training tool and a more timely and effective way to determine the next steps for the patients and their families. The equipment was set up at the University of Minnesota site in September 2015. Clinical and support staff have been trained on equipment. We plan to have our first group of real-time consultations this spring. The equipment is portable and will be suitable for use at various sites served by the clinical staff working with Dr. Jeffrey Wozniak including a site in a remote area of the state of Minnesota. On May 10th I will be giving a talk in Minneapolis to physicians working at remote sites in Minnesota which will give me an opportunity to talk to them about setting up a telemedicine site at one of their clinics.

In addition we have had discussions with Dr. Dan Calac who is the Medical Director of the Indian Health Council (IHC) clinic in Northern San Diego County. The clinic provides services to the North San Diego County reservations of Inaja-Cosmit, La Jolla, Los Coyotes, Mesa Grande, Pala, Pauma, Rincon, San Pasqual, and Santa Ysabel. Our discussions have been positive and we hope to use telemedicine to examine approximately one-hundred children as part of a Native-American prevalence study of FASD. The children will be evaluated at the IHC Clinic with a hand-held camera that we have previously purchased. The images will be sent back to me in real-time in San Diego.

Aim #4: Circumstances in Ukraine where we had planned to do this study have made this aim impossible to carry out. We are not able to collect information in the project timeframe to complete the aim.

3. For this reporting period (April 1, 2015 - present), 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?

At most sites in the Ukraine and in the U.S. where I have seen subjects and controls I have been extensively involved in training and professional development. Pre- and post-doctoral students in Psychology as well as Pediatricians, Geneticists and Neonatologists virtually always attend my diagnostic clinics at the various CIFASD sites.

The DVDs that I have made are specifically designed to train physicians and other health care providers to diagnose FASD. They are being piloted by the San Diego Chapter of the American Academy of Pediatrics, which includes 500 pediatricians over the next month.

The telemedicine project has enormous possibilities to train physicians and other health care providers about diagnosis of FASD particularly in underserved area.

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

I give talks in Southern California on a weekly basis on FASD to Community Groups, Women's Auxiliaries, Schools, Lawyers and Judges, Clinician groups and Academicians. At these

meetings I talk about the new things that are being learned about FASD through the programs that I am specifically involved in as well as other observations that are coming out of CIFASD.

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

Specific Aim #1 (To assure consistency as well as accuracy in recognition of Fetal Alcohol Spectrum Disorders at all CIFASD project sites throughout the world) relates to the aims of all other clinical projects in the CIFASD consortium. Without an accurate diagnosis it is impossible for the clinical projects to confidently interpret their data.

7. What do you **plan to do for the next reporting period** (March 1, 2016 - April 1, 2017) to accomplish your project's specific aims?

Aim #1: I will continue to see children ascertained at all CIFASD sites.

Aim #2: I will evaluate the extent to which the 500 pediatricians from the San Diego Chapter of the AAP feel the DVD has been a help to them in better assessing children for a diagnosis of FASD. Thereafter, if the NIAAA believes it appropriate, I would like to disseminate the DVD to members of the AAP throughout the U.S.

Aim #3: I will begin Phase II implementation at the University of Minnesota and will proceed with implementation at a remote clinic in western Minnesota associated with the University of Minnesota and with the Indian Health Council Clinic in Northern San Diego County.

8. CIFASD Phase III ends in May of 2017. **What specific aims do you see your project addressing in the upcoming competitive renewal?**

#1 Continuing to see children ascertained at all CIFASD sites.

#2 I would like to take up the issue of stigma as it relates to both biologic and adoptive mothers and effected children. There is no question that families and children effected with this disorder are stigmatized. That does not need to be established. Furthermore there is no question in my mind that we need scientists with special expertise in the field of stigma to attack this problem. I have identified such an individual and would like to collaborate with him on a hypothesis driven project to identify how to help effected individuals and their parents overcome the incredible discrimination and loss of status that results in devaluing them, rejecting them and excluding them.

9. What do you believe the **hot areas of FASD research** to be?

- Prevention
- Intervention and treatment
- Early diagnosis
- Stigma
- Low dose drinking behavior
- Nutritional risk factors
- Genetic risk factors
- Biologic markers
- Epigentics
- Paternal exposures

10. Publications [Accepted & In Press]

NIH Public Access Compliance	Citation
Complete	Coles CD, Kable JA, Keen CL, Jones KL, Wertelecki W, Granovska IV, Pashtepa AO, Chambers CD. Dose and Timing of Prenatal Alcohol Exposure and Maternal Nutritional Supplements: Developmental Effects on 6-Month-Old Infants. Matern Child Health J. 2015 Dec;19(12):2605-14. PubMed PMID: 26164422; NIHMSID: NIHMS709768; PubMed Central PMCID: PMC4644455.
Complete	Migliorini R, Moore EM, Glass L, Infante MA, Tapert SF, Jones KL, Mattson SN, Riley EP. Anterior cingulate cortex surface area relates to behavioral inhibition in adolescents with and without heavy prenatal alcohol exposure. Behav Brain Res. 2015 Oct 1;292:26-35. PubMed PMID: 26025509; NIHMSID: NIHMS705086; PubMed Central PMCID: PMC4558293.
Complete	Kable JA, Coles CD, Keen CL, Uriu-Adams JY, Jones KL, Yevtushok L, Kulikovsky Y, Wertelecki W, Pedersen TL, Chambers CD. The impact of micronutrient supplementation in alcohol-exposed pregnancies on information processing skills in Ukrainian infants. Alcohol. 2015 Nov;49(7):647-56. PubMed PMID: 26493109; NIHMSID: NIHMS725623; PubMed Central PMCID: PMC4636447.
Complete	Gautam P, Lebel C, Narr KL, Mattson SN, May PA, Adnams CM, Riley EP, Jones KL, Kan EC, Sowell ER. Volume changes and brain-behavior relationships in white matter and subcortical gray matter in children with prenatal alcohol exposure. Hum Brain Mapp. 2015 Jun;36(6):2318-29. PubMed PMID: 25711175; NIHMSID: NIHMS665461; PubMed Central PMCID: PMC4631525.

11. Publications [In Preparation & Submitted]

Kable JA, Coles CD, Jones KL, Yevtushok L, Kulikovsky Y, Wertelecki W, Chambers CD, and the CIFASD. Cardiac Orienting Responses Differentiate the Impact of Prenatal Alcohol Exposure in Ukrainian Toddlers. Alcohol Clin Exp Res, Submitted Feb. 2016

12. Poster Abstracts and Presentations

Nothing to report.

Principal Investigator(s): Christina Chambers
Institution(s): University of California, San Diego
CIFASD Project Title: Early Identification of Affected Children and Risk Factors for FASD in Ukraine
Grant Number: 5 U01 AA014835-12

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?

Table 1. Recruitment

Measure	Sample Size Goal by End of Funding	Sample Size Achieved as of 2/1/16
New pregnant women enrolled – alcohol exposed	60	88
New pregnant women enrolled – alcohol unexposed	60	74
Toddler visit at 2 years for growth and biological sampling	240	188
Preschool visit at 3.5-5 years for growth, biological sampling and neurobehavioral testing battery	240	202
Blood sampling of children in at 2 year visit		39
Blood sampling of children at 3.5-5 year old visit		64

Aim 1. We have tested 78% of the target 240 sample size of preschool children at both study sites with the preschool testing battery. After exclusions, we have performed preliminary unadjusted analysis on an N of 189 children (No/Low Alcohol with no multivitamin intervention N = 42; No/Low Alcohol with multivitamin intervention N = 68; Alcohol with no multivitamin/mineral intervention N = 40; Alcohol with multivitamin/mineral intervention N = 38). We collapsed the multivitamin/mineral intervention group with the added choline group for this analysis as our

previously published findings from this study on the Bayley Scales of Infant Development at 6 months did not show an additional benefit of choline supplements. Children were tested at a mean age of 3.97 Years (SD: 0.34).

Differential Ability Scales, 2nd Edition, Preschool Version, Nonverbal subtests and Summary scores, were used to measure cognitive processing. On both Pattern Construction ($F_{(1,184)}$=6.35, p<.01) and Picture Similarities ($F_{(1,185)}$=3.88, p<.05), negative effects of prenatal alcohol exposure could be measured and this was reflected in the Spatial Standard Score ($F_{(1,185)}$=2.66, p=.1) and the Nonverbal Cluster Standard Score ($F_{(1,185)}$=2.14, p=.14) as well, although these are only trends due to the preliminary nature of this analysis. Alcohol effects were noted on measures of Executive Functioning and Working Memory (Corsi Block Forward Total Correct, $F_{(1,170)}$=3.98, p<.05); Delayed Attention Total Errors, $F_{(1,178)}$= 4.46, p<.04, and Speeded Naming, $F_{(1,133)}$=10.8, p<.001). In addition, a number of other tasks showed trends for alcohol effects but the analysis is, as yet underpowered. Trends for effects of the multivitamin/mineral intervention were noted on several measures including the Corsi Blocks Forward Span (Working memory), Delayed Attention and Speeded Naming. Finally, interactions were noted on the Corsi Block Backward Total Correct ($F_{(1,123)}$=5.97, p<.02) , AB Total Correct (Memory, Executive Functioning, $F_{(1,183)}$=3.68, p=.06), AB Perseveration Errors ($F_{(1,183)}$=1.66, p=.19). These results are encouraging but interpretation will require an adequate sample size.

Executive Functioning was also measured using the BRIEF, a parent report measure. On this standardized measure, parents reported effects of alcohol on Inhibit, Emotional Control, Working Memory and ability to Plan/Organize. All of the summary score Indices on this measure were found to be negatively influenced by alcohol exposure. The pattern of outcomes associated with multivitamin/mineral intervention was in the positive direction but did not achieve significance as yet, demonstrating the need for analysis when the full sample is available. Child Behavior and Emotional Problems were measured using the Ukrainian Child Behavior Checklist also completed by the parent. On this measure, alcohol effects were found on the following Behavior Clusters: Anxiety/Depression ($F_{(1,171)}$=5.67, p<.02), Attention ($F_{(1,171)}$=6.5, p<.01) and Aggressive Behavior ($F_{(1,171)}$=9.27, p<.003). Withdrawn Behavior and Somatic Complaints approached significance. Summary Scores all showed significant effect of alcohol group: Internalizing ($F_{(1,171)}$=9.99, p<.002), Externalizing ($F_{(1,171)}$=5.01, p<.03) and Total Behavioral Problems ($F_{(1,171)}$=8.29, p<.004). There were trends for an interaction between Alcohol Group and multivitamin/mineral intervention group on Anxiety/Depression, Withdrawn Behavior, Somatic Problems, and Attention, as well as Total Behavior Problems. The interaction was significant for Externalizing Behavior ($F_{(1,171)}$=7.82, p<.006) with those in the Control group who received the vitamin intervention showing significantly fewer behavior problems.

The target sample size is 60 in each group for this Aim. We believe we are on target to complete this objective within the next five months as originally projected, which will give us sufficient sample size to detect similar effect sizes as were already seen on the Bayley. The overall significance of any findings related to the prenatal multivitamin/mineral intervention in children 3-5 years after birth will be determined when this analysis is completed.

In addition to the preschool testing battery, children at both sites have continued to be evaluated using the Cardiac Orienting Response (COR) paradigm. One publication and one submitted manuscript have already addressed the vitamin intervention using this measure in infants. Key findings indicated that choline supplementation combined with multivitamin/mineral supplementation had a beneficial effect on visual habituation in both alcohol exposed and unexposed infants, and this finding was consistent with the association of habituation with positive change in choline levels in maternal blood samples over the course of gestation. The overall significance of this finding was that maternal nutritional status and maternal choline

supplementation in pregnancy was related to better performance of the offspring on the COR in infancy, and thus could be an important intervention. The COR paradigm has been modified for preschool age children and is being administered to children in that age group in the Ukraine cohort; however, the sample size is not yet sufficient for analysis. We believe we are on target to recruit sufficient sample size to be able to evaluate the preschool sample on the COR this year.

Aim 2. A total of 39 toddler and 64 preschool blood samples have been collected and shipped to UC Davis, with varying quantities of sample available for analyses. Plasma samples from control children (N=31), alcohol-exposed children with FASD (N=7) or without FASD (N=8); (overall mean age 3.6 ± 0.9 y) were analyzed at UC Davis for select micronutrients. Using ANCOVA, preliminary data showed no differences in plasma levels of soluble transferrin receptor, soluble transferrin receptor-ferritin index, high-sensitivity C-reactive protein, homocysteine, and cortisol among the three groups. Children displaying features of FASD had significantly lower plasma ferritin levels (1.17±0.10 log ng/mL) compared to control children (1.49±0.05 log ng/mL, p=0.028) but were not different than unaffected children with prenatal alcohol exposure (1.38±0.09 log ng/mL).

Toddler and preschool growth and eating behaviors/eating style have been assessed for children at both sites at 2 and 3.5-5 years of age. Analysis has been completed for 201 children with significant findings on measures of Food Responsiveness and Satiety specifically in alcohol-exposed children and significant positive associations with these altered eating behavior measures and BMI growth trajectory. These data suggest that prenatal exposure to alcohol may impact eating behaviors which in turn may be related to altered growth trajectories that are evident in early childhood. The overall significance of this finding is that postnatal growth deficiency, which has been considered a hallmark of FASD, may not be universal, and a subset of prenatally exposed children may instead be at increased risk of overweight or obesity as they enter adolescence. If this pattern of altered growth is evident in toddlers or preschoolers and is related to the child's eating style, interventions can be initiated to help address this future risk.

With respect to blood measures in children, this has been a significant challenge in the two recruitment sites, both in terms of getting parent agreement to do any child blood sampling at all, and in terms of getting sufficient quantity to accomplish this aspect of Aim 2. Prioritization of available samples for the various desired uses is being explored, and methods and strategies for encouraging higher compliance by mothers in the study are being reviewed.

The eating behavior and growth analysis with the present sample size is sufficiently powered to proceed to publication which is being led by Dr. Kerri Boutelle at UCSD. Child growth measures are currently being examined in relation to prenatal maternal nutritional status from blood measures and based on multivitamin/mineral intervention status.

Aim 3. Dr. Miranda's lab was provided with 136 maternal samples from 68 pregnant women enrolled in the Ukraine cohort. Assessment was completed for plasma miRNA profiles (752 unique human miRNAs) for mid and late pregnancy in these women. This included 22 women with known moderate to heavy alcohol consumption with FASD affected infants (Group A), 23 women with known moderate to heavy alcohol consumption with apparently unaffected infants (no physical features and normal Bayley scores) (Group B), and 23 control women (no or low alcohol exposure) (Group C). The objective, to complete miRNA assessments, extract data and share among the investigators was accomplished by December 2015. Current objectives are to analyze the data more thoroughly and create predictive statistical models.

Our preliminary analysis indicates that there are statistically significant differences in miRNA expression between groups, after adjusting for multiple comparisons. A random forest analysis of Groups A and C, with the inclusion of clinical variables including maternal age, smoking and

socioeconomic status, achieves an Overall Misclassification Rate of 11.96%. This means that ~88% of samples in group A and C can be correctly assigned to their respective groups. In contrast, the random forest approach has a misclassification rate of 33.7% for the Group A vs. B comparison and a misclassification rate of 14.3% for a Group B vs. C comparison. Several specific miRNA pathways are represented in the specific miRNA's that are most discriminating in each of these comparisons.

The overall significance and health impact of these findings is important. Those specific miRNA's and pathways that differentiate groups A or B from group C may represent a marker of substantial prenatal alcohol exposure. In addition, these findings may also shed light on the mechanisms whereby alcohol affects fetal development. Separately, to the extent that specific miRNA's and pathways differentiate groups A from B, these findings can potentially contribute to understanding of individual susceptibility or resilience to the prenatal effects of alcohol, and may help predict during fetal life those infants that should be targeted for early intervention.

Currently, analyses are being completed to proceed to publication. The child samples collected under Aim 2 above are available and can be paired with maternal samples as a next direction for the miRNA work.

Aim 4. We have provided maternal samples to Dr. Joanne Weinberg's developmental project and analyses are currently being completed and are presented in her developmental project progress report. Addition of the clinical covariates and review of the data and results are planned for February 22-23, 2016 when the UBC team will visit San Diego. The data are being shared with Dr. Miranda as they evolve, and the same statistical team at UCSD is working on both datasets. There is overlap between the two sample sets that Drs. Miranda and Weinberg are analyzing for 48 of the mothers, so a combined analysis following each of the independent analyses is planned.

We continue to provide 3D images to Dr. Tatiana Foroud. The 3-D camera stopped functioning in early 2015, so we delivered a new camera in May of 2015, and images continue to be captured and uploaded to Indiana University, with interaction with IU regarding quality of images after each upload.

We have provided samples to Dr. Dipak Sarkhar for his developmental project. The analyses have been completed and findings with respect to epigenetic markers of stress are consistent with those he has noted previously in animal models and in children with FASD through CIFASD. These findings may also be relevant to those of Drs. Miranda and Weinberg.

We are prepared to do the exploratory gene sequencing analyses on mother and child samples from the Ukraine cohort. All samples sufficient to accomplish this are now housed at UCSD (maternal buffy coats and child buccal swabs and/or saliva), and we are ready to proceed. We believe the strategy for the consortium in terms of target genes, e.g., those involved in choline requirements, should be determined collaboratively before proceeding to expend resources to do this analysis.

As stated in previous progress reports, the telemedicine portion of this Aim was relocated by the Dysmorphology Core to the Minnesota CIFASD site.

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

3U01AA014835-12S1 Diversity Supplement to Diego Mesa, a predoctoral student in the School of Engineering at UCSD. Mr. Mesa's project in the first year beginning in August 2015 is focused on developing an individual predictive model for the COR data. Currently, group differences can be detected on the COR but this measure has not previously been developed

as a tool for individual prediction. The goal of Mr. Mesa's project is to utilize the COR data on 6 month olds, along with clinical and other data, to predict individual performance on the Bayley at 12 months and on the DAS at 3.5-5 years with acceptable sensitivity and specificity. He is new to the field of alcohol research, and is completing extensive background reading as well as presenting his progress on bi-monthly conference calls along with Dr. Julie Kable and Dr. Todd Coleman who is his PhD advisor in Engineering at UCSD. The results of the first stage of the predictive model are in preparation for a manuscript and were submitted to the 2016 RSA meeting as an abstract.

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

Two postdoctoral scholars have worked on this project over the last year. They both have IDPs as required by our institution. These are developed in collaboration and consultation with the mentor and are reviewed and revised on at least an annual basis. Both have required academic enrichment activities such as attending seminars and courses. The two fellows have worked on data analysis over the last year, and one has a submitted manuscript and the other has a manuscript in preparation for submission, both of which involve data from this study. In addition, three pre-doctoral students (two at UC Davis and one from Emory) have worked with data from this study. At their institutions no IDPs are required at the pre-doctoral level. However, we have provided both trainees with in-person and remote mentoring on the study design and statistical analysis techniques appropriate for the sample.

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

In the last year, have presented results of the nutritional intervention at a pre and postdoctoral visiting scholar event at the University of Wisconsin, at an FASD training in Krakow, Poland, and as requested through NOFAS.

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

This project has interacted with the Dysmorphology Core for physical examinations of children, with Drs. Foroud and Hammonds' 3D imaging project, and with the developmental projects of Drs. Sarkhar and Weinberg.

7. What do you **plan to do for the next reporting period** (March 1, 2016 - April 1, 2017) to accomplish your project's specific aims?

We plan to finish testing the full preschool sample and complete the final analysis. We plan to increase our efforts to collect blood samples from children, supplementing the sample with the newly recruited mothers and their children who are now two years or age or older. We plan to complete the analyses of Aim 3 and proceed to publication, and to finalize the outstanding publications in preparation or currently under review.

8. CIFASD Phase III ends in May of 2017. **What specific aims could you see your project addressing in the upcoming competitive renewal?**

- As the cohort will enter adolescence in the upcoming renewal period, there is a unique opportunity to follow longitudinal growth from prenatal to adolescence. There is also an opportunity to examine early markers of adult disease.
- As the cohort can continue to enroll new pregnant women, there is an important opportunity to replicate and enhance the miRNA and markers of inflammation findings in mothers and their children from birth to adolescence. This would include markers of stress in mothers and children. As both the miRNA and markers of inflammation findings may relate directly to placental function, there is also an opportunity to collect placental samples in this cohort.

- The mothers and children in the cohort can be readily assessed at multiple time points and ages for their microbiome status. This would include collection of fecal samples, and skin swabs. Archived urine samples from mothers and children in the cohort that are currently housed at UCSD can also be used for this purpose.
- Interventions
- Identify patterns of resilience across the CIFASD samples. This could be an early childhood kind of score that would help predict the trajectory for children and opportunities for intervention.

9. What do you believe the **hot areas of FASD research** to be?

- Identification of high-risk pregnancies; early diagnosis
- Interventions including treatments

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

NIH Public Access Compliance	Citation
Complete	Coles CD, Kable JA, Keen CL, Jones KL, Wertelecki W, Granovska IV, Pashtepa AO, Chambers CD. Dose and Timing of Prenatal Alcohol Exposure and Maternal Nutritional Supplements: Developmental Effects on 6-Month-Old Infants. Matern Child Health J. 2015 Dec;19(12):2605-14. PubMed PMID: 26164422; NIHMSID: NIHMS709768; PubMed Central PMCID: PMC4644455.
Complete	Kable JA, Coles CD, Keen CL, Uriu-Adams JY, Jones KL, Yevtushok L, Kulikovsky Y, Wertelecki W, Pedersen TL, Chambers CD. The impact of micronutrient supplementation in alcohol-exposed pregnancies on information processing skills in Ukrainian infants. Alcohol. 2015 Nov;49(7):647-56. PubMed PMID: 26493109; NIHMSID: NIHMS725623; PubMed Central PMCID: PMC4636447.

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

Kable, J.A., Coles, C.D., Jones, K.L., Yevtushok, L., Kulikovsky, Y., Wertelecki, W., Chambers, C.D. and the CIFASD. Cardiac Orienting Responses Differentiate the Impact of Prenatal Alcohol Exposure in Ukrainian Toddlers ACER. Submitted February 2016; CIFASD manuscript submission template completed February 2016.

Bandoli, G., Coles, C.D., Kable, J.A., Wertelecki, W., Granovska, I.V., Pashtepa, A.O. Chambers, C.D. and the CIFASD Assessing the independent and joint effects of un-medicated prenatal depressive symptoms and alcohol consumption in pregnancy and infant neurodevelopmental outcomes. ACER. Submitted February 2016; CIFASD manuscript submission template completed February 2016.

Miranda R, MiRNA's in maternal plasma as predictors of alcohol exposure and affected children. CIFASD concept proposal template completed 2016.

Fisch, K. Differential methylation patterns in pregnant drinkers whose children have FASD; CIFASD concept proposal template completed 2015.

Sowell K. Implications of altered IL-6 concentrations on infant outcomes of children with prenatal alcohol exposure. CIFASD concept proposal template completed 2015.

Boutelle, K. Eating behavior patterns in children with prenatal alcohol exposure. CIFASD concept proposal template completed 2015.

Weiss, L. Maternal iron status in alcohol-exposed mothers and fetal growth. CIFASD concept proposal template completed 2015.

Montag, A. Ultrasound markers of physical features of FASD. CIFASD concept proposal template completed 2015.

12. **Poster Abstracts and Presentations**

Presentations:

Visiting Professor invited speaker, University of Wisconsin, September, 2015.

NeuroDevNet invited speaker, Ottowa, Canada, September, 2015.

FASD Training invited speaker, Krakow, Poland, October, 2015.

Poster Abstracts:

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. RSA, June 2015.

Chambers, C.D., Yevtushok, L., Zymak-Zakutnya, N., Wertelecki, W., Schaefer, J., Boutelle, K. Differential eating behavior patterns in children with moderate to heavy prenatal alcohol exposure. RSA, June 2015.

Principal Investigator(s): Tatiana Foroud and Peter Hammond
Institution(s): Indiana University; University College London/University of Oxford
CIFASD Project Title: Craniofacial Dysmorphism & Fetal Alcohol Exposure
Grant Number: 5 U01 AA014809-12

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

G1) 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.
G2) Recruit and analyze facial imaging data from very young populations to develop a screening tool that accurately identifies high risk individuals for future intervention.
G3) Combine face images, neurobehavioral data and brain images to identify common pathways and hence improve diagnosis of prenatal alcohol exposure.
G4) 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.
G5) Extend preliminary genetic studies through collection of DNA samples for new subjects and focused analysis to replicate candidate genes identified in basic science components.

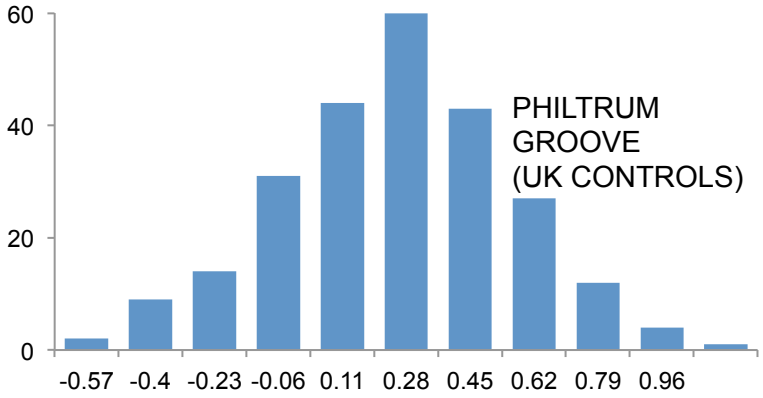
2. What was **accomplished** under these goals?

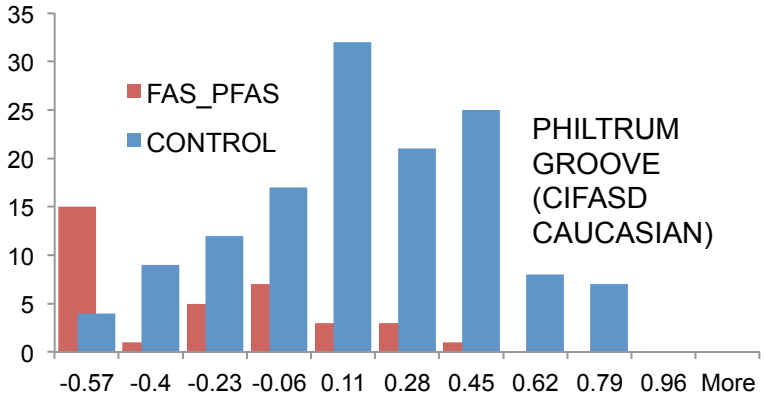
G1&G2 GROOVE: new objective measure of philtrum smoothness
A major effort has been further testing and development of algorithms/software for delineation and measurement of raw and normalised curvature difference to identify philtrum smoothness. Previously, we used raw/normalized surface displacement to determine differences/similarities between individuals and mean faces. Surface displacement reflects face size difference which contributed to accurate control-FAS discrimination - because of effects of PAE on growth. However, for heavy exposure individuals (HE) with known PAE and facial dysmorphism too subtle for clinical detection, size difference can overwhelm subtle philtrum/upper lip effects. Therefore, we introduced curvature difference, which is less responsive to size difference, to delineate general curvature across a face as well as curl and groove curvatures which are particularly useful for detecting philtrum smoothness.

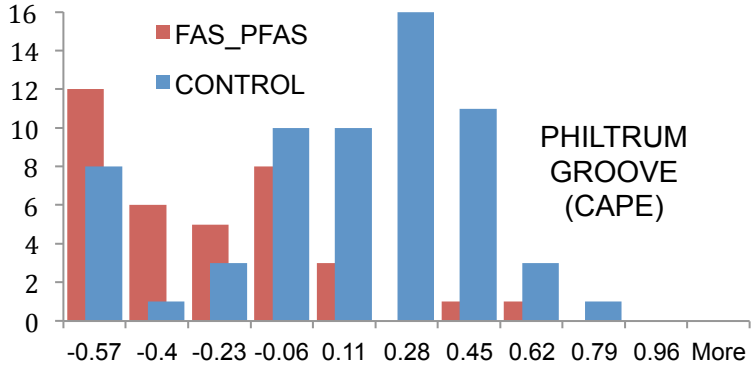
Curvature difference has both advantages and disadvantages over surface difference in terms of image preparation before analysis and sensitivity to image quality. Previous surface difference methods employed dense surface modelling (DSM) techniques which required a proband image and a cohort of clinically categorized images to be (manually) annotated with 22 or so anatomical landmarks before analysis even started. Raw, general surface curvature of a proband image is visualized immediately with no annotation and is of immediate clinical use. Raw groove and curl curvature, requiring only exocanthi, subnasale and upper lip centre landmarks, are also clinically useful. Normalised curvature requires a DSM to be built as with surface difference techniques. Signature graphs have also been extended to deal with curvature.

As more sophisticated curvature based techniques were developed, e.g., for computing philtrum groove volume, the sensitivity of curvature to “noise” adversely affected reliability of its calculation. For example, holes and spikes can arise during image capture, simply due to perspiration on the face or saliva on the lips. We also found many images had been captured with poor camera calibration resulting in seams or rough patches, often across the upper lip. Such surface “noise” is easily overlooked if the camera operator doesn’t remove the texture/appearance to inspect the underlying surface. The issue is how to fix holes and spikes without affecting philtrum smoothness. The automatic calculation of philtrum volume was adversely affected in 10% to 50% of images tested depending on the dataset. Images on which

the philtrum volume calculation has been tested so far include: UK controls (400); CIFASD (250); CAPE (200); COFASP (110); UKRAINE (86); PASS (1200). To overcome some problems, we have produced a tool for manually outlining the philtrum to enable automatic calculation of its volume. The figures below show GROOVE (*log(philtrum-vol/philtrum-length)*) distribution for several datasets.







Periorbital effects at 1 yr of age due to substantial nicotine but no alcohol exposure

Of the 1mth-1yr pairs of 3D photographs provided by the PASS network, 600 (60%) have been landmarked and subject to DSM & curvature based analyses. Using alcohol and tobacco exposure data provided by DM-STAT, 100 or so individuals were designated as controls using maternal declarations of no alcohol/no smoking exposure before and during pregnancy. In addition, ~200 non-drinking mothers declared heavy smoking before and in pregnancy. Significant difference in PFL and eye separation has been identified in their infants at 1yr of age.

G3 Face-brain-psychometry analysis & corpus callosum/ethnicity shape discrepancies

Previously, in a Cape Coloured cohort of undiagnosable but alcohol exposed individuals, correlations were identified between face shape and psychometric performance. This was not repeatable in a Caucasian cohort which may be because the psychometric tests did not directly match those of the Cape cohort. Face-corpus callosum shape correlations were identified as statistically significant. These have now been extended to the caudate nucleus. A combined face/caudate nucleus/corpus callosum model is currently being generated.

During the face-brain analysis, significant (>90%) discriminating differences were identified in corpus callosum shape for control groups from two sites where each was dominated by a different ethnic background (African-American versus Caucasian-Hispanic). The discrepancy remains unexplained. As a result, Mike Suttie has pushed the images through the UCL atlas based segmentation pipeline. The resulting segmentations appear to be cleaner and smoother. The analysis will be completely redone with the revised segmentations.

G4 Differential diagnosis of FAS from CdLS, 22q11del and Williams syndromes

From PH's previous studies 385 3D facial photographs were available of 170 controls (3.1 to 17.9 yrs), 33 individuals with FAS (6.4-17.8 yrs), 50 with Cornelia de Lange syndrome (CdLS) (5.1-17.4 yrs), 67 with 22q11 deletion syndrome (VCFS) (4.1-16.6 yrs), and 65 with Williams syndrome (4.2-17.7 yrs). Simultaneous closest mean comparison of FAS to the three syndromes, for the whole face, using drop-one-out unseen testing was 100% accurate in classifying all 215 syndromic individuals (Table 1). For the nasal region alone, accuracy was 97.5%. When restricted to mid-line facial profile or philtrum alone, overall classification accuracy was 78.6% and 73.6% respectively. Interestingly, of the three differential diagnoses, Cornelia de Lange was the syndrome most frequently misclassified as FAS, especially for the philtrum. Philtrum convexity in Cornelia de Lange syndrome is the most likely explanation for this.

	CLASSIFICATION (% of syndrome cases – 0% left blank)							
	FULL FACE WITHOUT EARS				**MID-LINE FACIAL PROFILE**			
DIAGNOSIS	**CDLS**	**FAS**	**22q11**	**WS**	**CDLS**	**FAS**	**22q11**	**WS**
CDLS (n=41)	100.0				75.6	12.2	2.4	9.8
FAS (n=33)		100.0			3.0	87.9	9.1	
VCFS (n=65)			100.0		1.5	12.3	81.6	4.6
WS (n=62)				100.0	9.7	9.7	8.0	72.6
	PHILTRUM				**NOSE**			
DIAGNOSIS	**CDLS**	**FAS**	**22q11**	**WS**	**CDLS**	**FAS**	**22q11**	**WS**
CDLS (n=41)	68.3	22.0	9.7		95.1	4.9		
FAS (n=33)		90.9	3.0	6.1		100.0		
VCFS (n=65)	4.6	13.9	78.5	3.0			100.0	
WS (n=62)	8.0	16.1	12.9	63.0		3.2	1.6	95.2

Table 1 Drop-one out closest mean comparison of FAS simultaneously to 3 differential diagnoses

G5 Facial image and DNA collection in CIFASD

Over the past year we have continued to obtain 3D facial images as well as DNA samples from individuals participating in CIFASD. A total of 250 new subjects provided a 3D image and longitudinal images were collected from other subjects. Two hundred new DNA samples were also collected this year. In a collaboration with PASS, 3D images were also obtained from 175 subjects in the Plains (197 total images).

As described in the Developmental Project, samples have been sent for SNP genotyping and analyses have been performed in collaboration with Dr. Charness and Dr. Eberhart to evaluate the role of candidate genes.

Site	3D Images (# subjects)[1]	DNA[1]
San Diego[2]	435 (347)	458
UCLA/USC	104 (88)	176
Atlanta	300 (300)	418
Minneapolis	315 (309)	420
Ukraine	214 (185)	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	**4,283 (2,801)**	**1,697**

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

Excluding non-CIFASD and images collected outside the USA (i.e., excluding Ukraine, South Africa, and non-CIFASD individuals from Atlanta), there are 1,014 individuals with a 3D image. Phenotype data for these individuals is provided below. In summary, 212 individuals have data in only one domain, 506 have data for only 2 domains and 194 individuals have data in all 3 domains. There are 102 individuals with a 3D image and no other phenotypic data.

Individual has data:	in all domains	in only	in only	in only	for*	for*	for*
Dysmorphology	x	x	x		x		
Neurocognitive	x	x		x		x	
Brain	x		x	x			x
Total Number	**194**	**494**	**0**	**12**	**762**	**838**	**236**

* may have data in other domains.

3. **For this reporting period (April 1, 2015 - present), 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?** Mike Suttie continues to be registered for a part-time PhD at UCL.

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

Through talks and direct *pro bono* face evaluation of several children of adoptive families who were in contact following the screening of UK TV documentary “When Pregnant Women Drink Alcohol”. In one case, the face analysis convinced the local health authority to fund referral to the UK’s only specialist FASD clinic run by Dr Raja Mukherjee.

6. Describe your project’s **interrelation** with aims of the CIFASD consortium & CIFASD projects

Peter Hammond (10%) and Mike Suttie (20%) contribute to Dr. Kathy Sulik’s North Carolina mouse study. Mike Suttie works closely with Lindsay Wieczorek on mouse brain segmentation.

7. What do you **plan to do for the next reporting period** (March 1, 2016 - April 1, 2017) to accomplish your project’s specific aims?

Complete landmarking and exposure-face analysis of the PASS 1m-1yr pairs dataset; generate a face/corpus callosum/caudate nucleus triple component model and reattempt the correlation analysis with psychometric data; prepare face analysis software for distribution.

8. CIFASD Phase III ends in May of 2017. **What specific aims do you see your project addressing in the upcoming competitive renewal?** Development of smartphone apps and handheld devices to capture 3D facial images; Automated landmarking/evaluation of 2D/3D

images for facial effects of prenatal alcohol exposure; Combined data from genetics, facial and brain imaging and neurobehavioral data obtained in diverse subject populations to identify common pathways affected in fetal alcohol spectrum disorders; Use of 3D fetal ultrasound to detect developing facial, skeletal and neural structures and potential alcohol exposure effects.

9. What do you believe the **hot areas of FASD research** to be?

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

Nothing to Report.

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

Concept proposal forms for the 3 papers below were submitted in 2015:

- Earlier results demonstrating discrimination of FAS from 3 differential diagnoses postnatally have been linked to the recent demonstration of the potential for the prenatal mid-line facial profile to detect PAE facial characteristics in 3D ultrasound images in a paper aimed at influencing appropriate sonography for studies of FAS in 3D ultrasound images. The manuscript is complete and will be submitted once the PASS publications committee has validated it.
- Following an invitation from a genetics journal, a paper on face curvature analysis in FAS and genetic anomalies will be submitted by April 1st to appear in a special issue later in the year.
- A draft manuscript summarizing ethnic comparisons of PAE effects in Caucasian and Cape Coloured cohorts needs only a concluding section to be ready for submission.

12. **Poster Abstracts and Presentations**

May PH: American Psychiatric Association, Conference, Toronto, CANADA
Jun PH: RSA Conference, San Antonio, USA
Oct PH: Nuffield Dept of Obstetrics & Gynaecology, University of Oxford, UK
Oct PH: Department of Medical Genetics, Vilnius, LITHUANIA
Dec PH: Annual Developmental Biology Symposium, Oxford, UK
Jan PH: MSc Embryology Course, University of Oxford, UK
Feb PH: NOFAS-UK Conference, London, UK
Feb PH: British Neuropsychiatry Association Conference, London, UK

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 AA017122-09

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 one CIFASD psychometricians, Alexy Andrade. Alexy maintains his approval for neurobehavioral testing and has been evaluating CIFASD participants over the two years. To date, we have evaluated 65 (35 AE, 30 CON) participants at CHLA for the neuroimaging protocol. Of these, 24 (15 AE, 9 CON) have returned for the 2 year follow up appointments.
SDSU: A total of 59 CIFASD participants have been evaluated with neuroimaging and neurocognitive measures, and all neuroimaging data have been transferred to USC/CHLA. Of these, 15 (12 AE, 3 CON) have returned for the 2 year follow up appointments.
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. Of these, 16 (8 AE, 8 CON) have returned for the 2 year follow up appointments.
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, 38 have returned for the 2 year follow up appointments.

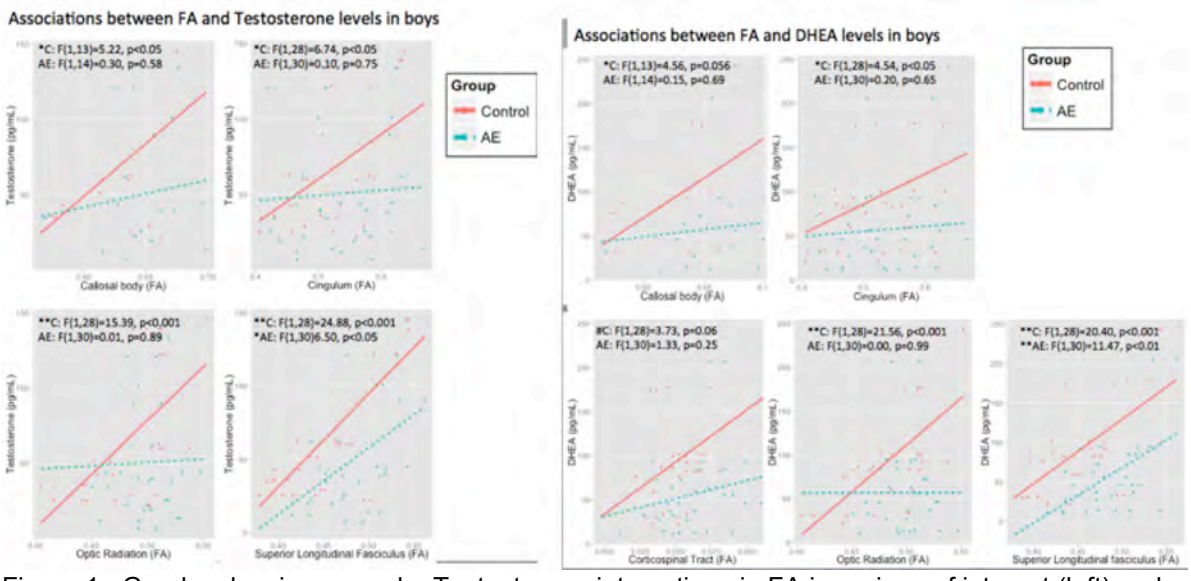


Figure 1: Graphs showing group by Testosterone interactions in FA in regions of interest (left) and DHEA by group interactions in similar regions of interest.

Brain Image Analyses: Studies and Results

USC/CHLA:

Associations between white matter microstructure and gonadal hormone levels are altered differently in boys compared to girls with fetal alcohol spectrum disorder.

In animal models, prenatal alcohol exposure (PAE) results in endocrine dysregulation and sexually dimorphic brain alterations. However, very little is known about sex differences and hormone levels among youth with Fetal Alcohol Spectrum Disorder (FASD). The present study aimed to fill this gap using diffusion tensor imaging (DTI) to assess: 1) whether or not sex differences in the impact of PAE on white matter microstructure exist; and 2) how gonadal hormones relate to alterations in white matter microstructure in PAE youth (n=61 youth (9.3-16 yrs; 49% girls; 50% PAE). Compared to Control youth, PAE girls exhibited *lower* FA values in 2 ROIs (p's<0.10), but PAE boys exhibited *higher* FA values in 5 ROIs (p's<0.05). Further, a loss of the typical positive relationship between FA and age was observed in 2 ROIs among PAE boys. For boys, positive associations were observed between FA and T, FA and DHEA and MD and T across 4 ROIs among Control boys, but only in 1 ROI among PAE boys (Figures 1 and 2). No significant associations between gonadal hormones and brain measures were observed among girls. **Conclusion**. These novel findings provide evidence that PAE results in: 1) sexually dimorphic effects on white matter microstructure; and 2) in a loss of typical hormone-brain associations in PAE boys. Further elucidation of endocrine alterations following PAE may inform novel endocrine therapies for individuals with FASD.

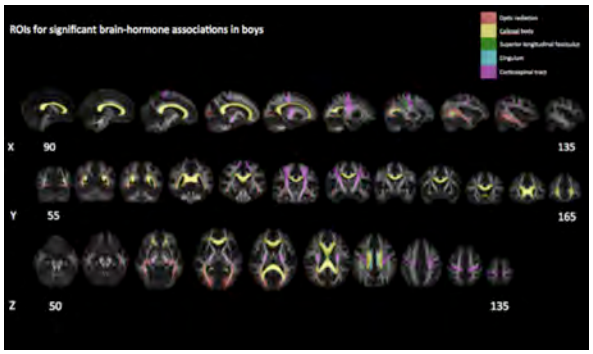


Figure 2. ROIs for significant brain-hormone associations in Control boys. Mean FA skeleton represented over standard mask (grey scale). Callosal body ROI (yellow); Cingulum (light blue); Corticospinal tract (pink); Optic radiation (red); superior longitudinal fasciculus (green). Jeulich max prob threshold 50 1mm ROIs.

SES by group interactions in brain surface area.

Structural MRI analyses using Freesurfer semi-automated parcellation and measurement from 186 participants (86 PAE & 94 controls) are described. Prior MRI studies have revealed that cortical surface area and thickness are altered in FASD, with some studies demonstrating increased thickness and others showing decreased thickness. In typically-developing children, socioeconomic status (SES) variables such as family income and education are associated with these structural variables. Therefore, we examined SES in the context of PAE. Results of these analyses reveal that Hollingshead SES index scores are positively associated with cortical thickness in post-central regions bilaterally in children with PAE. In control participants, a different pattern is seen, with positive associations primarily in the anterior temporal region. Statistically significant group by SES interactions in cortical thickness were observed in the same brain regions, suggesting that greater access to resources among those with higher SES may be differentially associated with brain development in children with PAE vs. controls. No significant relationships were seen with surface area. The data from control participants are consistent with findings from a recent large study of typical development that showed a strong relationship between family income and cortical thickness in anterior temporal and insular regions bilaterally (Fig 3). Taken together with previous longitudinal results of trajectories of change in the brain structure and function between PAE and controls, the data presented here suggest that the environment is an important factor in brain development in FASD. These findings support experience-dependent brain plasticity in youth with PAE and highlight the role that environment can play in FASD intervention.

Figure 3: Regions where indices of SES from the Holings Head measures correlate with cortical thickness in PAE and control participants.

Further analyses have been conducted in Dr. Sowell's laboratory to evaluate thickness:SES interactions with more sophisticated model fits including corrections for multiple comparisons (see Fig.4). In these analyses, we used ROIs of cortical thickness and a "path-of-least resistance" measure for various statistical models (i.e., linear and cubic models for age-correction). In general, cortical thickness better predicted SES measures and white matter surface area,

and, the cubic age-correction model was best for the exposed group. Further, SES by group interactions were significant in predicting cortical thickness in a few ROIs. We are in the process of refining these results, which may be presented by Jeff Wozniak **(given that Dr. Sowell will be attending the Human Brain Mapping conference in Geneva that conflicts with the dates of the RSA meeting)** at the RSA symposium this coming summer.

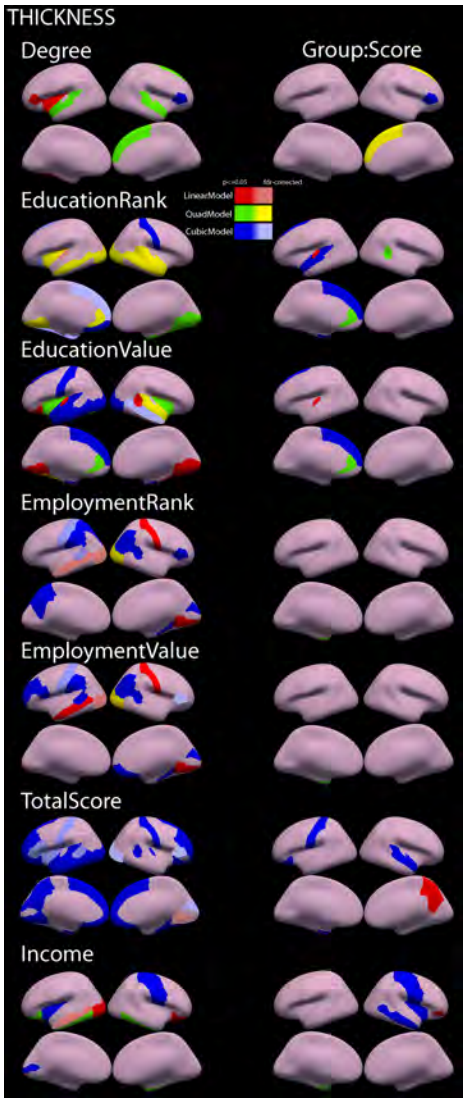


Figure 4: Statistical significance (FDR corrected) for cortical thickness in various ROIs for various statistical models.

UMN: Dr. Wozniak and his group are preparing a manuscript for submission entitled "Whole brain functional connectivity in children with prenatal alcohol exposure (PAE)". The analyses are now nearly complete and the paper is targeted for submission by March 15, 2016.

CIFASD Phase III data from all four clinical sites were used for these analyses. A total of 143 participants had good fMRI data (75 PAE, 68 Controls). Whole-brain functional connectivity metrics were examined, including: characteristic path length (CPL), mean clustering coefficient (MCC), Global Efficiency (GLOB), and Local Efficiency (LOC). At the group level (PAE vs. non-PAE), there were no significant differences in the means of these functional connectivity measures. However, a detailed analysis of individuals who showed atypical connectivity (>1 SD outside the typical range) revealed a very interesting set of findings (see Table).

Specifically, individuals with atypical connectivity were significantly more likely to have been prenatally-exposed to alcohol than to have been not exposed (2.4 times more likely for MCC and 27 times more likely for LOC). This suggests that taking a psychometric approach, analogous to what is done with neuropsychological data, may prove useful in identifying individuals with atypical brain functioning.

Even more interesting is the potential for applying functional connectivity measures to the problem of the "deferred" diagnostic group in CIFASD. Of the 126 participants who had good fMRI data and had a full dysmorphology evaluation, there were 12 diagnosed with FAS, 59 identified as non-FAS, and 55 deferred. Looking only at the deferred group of 55, we identified 15 individuals who had atypical functional connectivity on one or more measures. **As broken out in the Table, 100% of those 15 individuals who had atypical MCC, GLOB, or LOC were known to have been prenatally exposed to alcohol. There were no false positives** – in other words, the functional connectivity measures did not identify anyone from the control group.

Lastly, individuals who have atypical MCC, GLOB, and LOC show significant neurocognitive deficits relative to those with typical functional connectivity. Differences are seen in global cognitive functioning, processing speed, aspects of working memory, and ADHD-behavior.

These data suggest that functional connectivity abnormalities are relevant to PAE. Although the measures may not be sensitive at the group level (PAE vs. non-PAE), they are sensitive within a sub-group of individuals already identified as "suspect" on the basis of dysmorphology. Importantly, the data suggest a potentially very high degree of specificity when both types of data are considered together.

n (% within network measure)	PAE (n = 37)	Control (n = 18)	Chi-square, sig.
Characteristic Path Length (CPL)			
Typical	28 (63.6%)	16 (36.4%)	
Atypically high	9 (81.8%)	2 (18.2%)	x^2 =1.32, p=.307
Mean Clustering Coefficient (MCC)			
Typical	25 (58.1%)	18 (41.9%)	
Atypically high	12 (100%)	0 (0%)	x^2 =7.47, p=.005
Global Efficiency (GLOB)			
Typical	30 (62.5%)	18 (37.5%)	
Atypically low	7 (100%)	0 (0%)	x^2 =3.90, p=.051
Local Efficiency (LOC)			
Typical	25 (58.1%)	18 (41.9%)	
Atypically high	12 (100%)	0 (0%)	x^2 =7.47, p=.004

Table 1: Functional Connectivity Results.

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 structure, further emphasizing potential impact for interventions targeting deficiencies in the developmental environment could impact change over time.

SDSU: Dr. Mattson and colleagues examined neural correlates of memory function in children (ages 10-16y) with prenatal alcohol exposure and controls. An abstract on a portion of this study, examining surface area, was submitted to RSA. The results of that analysis showed differential relations between verbal learning measures from the CVLT-C and surface area of left entorhinal cortex and left and right parahippocampal cortices. Better CVLT-C performance related to increased cortical surface area in the AE group but decreased surface area in the CON group. These results are illustrated in **Dr. Mattson's progress report**. Analyses are ongoing for additional neural measures (volume and cortical thickness); we hope to submit a paper by 6/15/16.

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. Shantanu Joshi was awarded a K25 to pursue work (Joshi PI: K25 AA024192-01, Sowell Primary Mentor) to find quantitative relationships between the anatomy of the brain from MRI, and that of the face from 3D photographs of children affected by fetal alcohol spectrum disorder (FASD) caused due to prenatal alcohol exposure. A critical outcome of this research is expected to reveal diagnostic effects for children who have neurocognitive deficits but for whom the facial dysmorphology is not directly visible, and will have a significant impact for early detection and possible intervention strategies for FASD in the nation as well as all over the world, wherever alcohol is consumed by mothers during pregnancy. Moreover, this research will integrate methods from engineering and neuroscience, and will develop a common scientific approach for analyzing diverse imaging modalities such as MRI images (brain) and 3D laser scans (face).

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

Our "Science and Society" manuscript published in January 2016 in the journal Trends in Cognitive Sciences has been well received by the scientific community. It will be featured by the National Institutes of Alcohol Abuse and Alcoholism (NIAAA) on their website, and, in the 3 weeks since it's publication, it has received an "Altmetric" score for online presence of 15, which is in the top 25% of all scientific articles, and at the 91st percentile relative to all other articles published within the same time frame. We are optimistic that this important article on the dangers of drinking during pregnancy will have wide societal impact.

6. Describe your project's **interrelation** with aims of the CIFASD consortium its other projects. As seen in description of our studies above, we have been interacting primarily with the Neurobehavioral project. We continue work on integrating Brain, Cognition, Face and genetics in work lead by Dr.s Foroud, Hammond, Charness, Mattson and myself.

7. What do you **plan to do for the next reporting period** (March 1, 2016 - April 1, 2017) 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). We will submit numerous manuscripts being prepared by Kristina Uban, and will continue our analyses on SES in FASDs for manuscript submission in the coming months.

8. CIFASD Phase III ends in May of 2017. **What specific aims do you see your project addressing in the upcoming competitive renewal?**

Aim 1: To measure change in brain function, structure and connectivity in brain regions important for the most predictive cognitive/behavioral measures relate to change in these cognitive and behavioral measures, and to measure developmental environmental measures and perhaps genotypes that narrow variability in brain-cognition-behavior. The ultimate idea here is to see if we can bolster brain wiring for better long-term outcomes.

Aim 2: Associations between brain and facial morphology. The face develops early in the first trimester; thus the greatest degree of dysmorphology, at least as shown in animal models, is produced by binge consumption of alcohol during the first trimester[5]. Facial dysmorphology provides insight into brain alterations occurring during this time. The goal here would be to see if facial morphology at one time point can be used to predict behavior/cognition and brain at a later time. Can this be used to help us understand who would benefit most from environmental changes (as determined in aim 1) before problems occur?

9. What do you believe the **hot areas of FASD research** to be?

Overview: Better diagnosis is one objective, integrating multiple forms of data. Knowing what is affected and WHEN is key because **the brain develops within the environment. The brain changes differently over time long after the in utero environment. How do we predict the variability around the mean? Genes? Environment? Individual differences, and factors that predict better adaptation are important.**
Understanding change in the brain and cognition over time during development, and how that relates to facial morphology from combined face brain analyses. Can we use facial data earlier in development to predict later brain and cognitive development, in hopes of earlier intervention for children who will have problems later in development? We should capitalize on neurocognitive measures, in the briefest battery possible (with may be different depending on the age of participants), assess change over time on these measures along with changes over time in the brain. The ultimate goal should be to understand environmental (SES, nutrition, family stress, educational environments, exposure to adversity, any interventions of any kind) to help understand inter-individual variability. If we could do that, we could work towards changes in the environment (most likely interventions of some sort, behavioral, cognitive, etc.) to improve adaptation of those most negatively affected.

- Longitudinal brain imaging
- Longitudinal neurocognition
- 3D facial combined with brain
- Measuring factors that contribute to inter-individual variability (environment, genetics) in hopes of interventions in the nearer future.
- Maybe capitalize on birth cohorts (i.e., PASS) where we can get a better idea of QFT and lots of other in utero measures to predict how the brain develops later. This is the R01 proposal I submitted (revision to be reviewed Feb 25, 2016), but, the down side is the homogeneity of that population, and can it really translate directly to our US samples?

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

NIH Public Access Compliance	Citation
Complete	Gautam P, Lebel C, Narr KL, Mattson SN, May PA, Adnams CM, Riley EP, Jones KL, Kan EC, Sowell ER. Volume changes and brain-behavior relationships in white matter and subcortical gray matter in children with prenatal alcohol exposure. Hum Brain Mapp. 2015 Jun;36(6):2318-29. PubMed PMID: 25711175; NIHMSID: NIHMS665461; PubMed Central PMCID: PMC4631525.
Complete	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. 2015 Dec;25(12):4761-71. PubMed PMID: 25092900; PubMed Central PMCID: PMC4635917.

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

Uban KA, Herting MM, Wozniak JR, and **Sowell ER**. Associations between white matter microstructure and gonadal hormone levels are altered differently in boys compared to girls with fetal alcohol spectrum disorder. *Psychoneuroendocrinology.*

Uban KA., Herting MM, Kan EC, Colby JB, Adnams CM, May PA, Narr KL, **Sowell ER**. Along-tract statistics reveals alterations in structural connectivity in adolescents with a fetal alcohol spectrum disorder. *J. Neuroscience.*

Gautam P, Uban KA, Adnams CM, May PA, Narr KL, **Sowell ER**. Effects of alcohol exposure, maternal education, and family income on brain volume changes during development. *Human Brain Mapping.*

Uban KA, Herting MM, **Sowell ER**. Hemispheric asymmetry in structural connectivity in adolescents with a fetal alcohol spectrum disorder. *Developmental Psychobiology.*

12. **Poster Abstracts and Presentations**

Uban KA, Herting M, Wozniak JR, **Sowell ER**, and the CIFASD. Associations between white matter microstructure and gonadal hormone levels are altered in youth with fetal alcohol spectrum disorders (FASD). Society for Neuroscience, Chicago, IL, 2015.

Bodison SC, Uban KA, and **Sowell ER**. White Matter Integrity, Balance, and Bilateral Motor Control in Adolescents with FASD. Human Brain Mapping Annual Meeting, Honolulu, HI, 2015.

Bodison SC, Herting MM, and **Sowell ER**. Sensorimotor Integration in Typically Developing Children and Those with Autism. Flux Congress Annual Meeting, Leiden, Netherlands, 2015.

Uban KA & **Sowell ER**. White matter organization/connectivity, executive functioning and hormones are altered in adolescents with FASD: implications for sex differences in the FASD brain. International Conference on Fetal Alcohol Spectrum Disorders, Vancouver, BC, 2015.

Uban KA, Herting MM, Wozniak JR, **Sowell ER**. Associations between white matter microstructure and gonadal hormone levels are altered in youth with fetal alcohol spectrum disorders (FASD). Human Brain Mapping Annual Meeting, Honolulu, HI, 2015.

Upcoming:

Doyle LR, Moore EM, Coles CD, Kable JA, **Sowell ER**, Wozniak JR, Riley EP, Mattson SN, & the CIFASD. Cognitive Predictors Of Communication Impairment In Youth With Histories Of Heavy Prenatal Alcohol Exposure. Research Society on Alcoholism, New Orleans, June 2016.

Gross LA, Moore EM, Coles CD, Kable JA, **Sowell ER**, Wozniak JR, Riley EP, Mattson SN, & the CIFASD. Temporal Cortex Surface Area Predicts Verbal Memory In Youth With Heavy Prenatal Alcohol Exposure. Research Society on Alcoholism, New Orleans, June 2016.

Cheney DC, Wozniak JR, Coles CD, Kable JA, **Sowell ER**, Riley EP, Mattson SN, & the CIFASD. Interaction Of Prenatal Alcohol Exposure And Postnatal Ses On General Cognitive Ability. Research Society on Alcoholism, New Orleans, June 2016.

Wozniak JR, Kan E, Mattson SN, Jones KL, **Sowell ER**, & the CIFASD. The Role Of Socioeconomic Status In Brain Development Among Children With Heavy Prenatal Alcohol Exposure. Research Society on Alcoholism, New Orleans, June 2016.

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 AA014834-12

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

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.

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.

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.

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**. As a results, we have made considerable progress on all 4 domains.

Table 1. Number of subjects tested

Neurobehavioral Progress 2012-2017				
Site	Goal Per Group	Alcohol-Exposed	Control	Contrast
SDSU Old	44	44	50	39
SDSU Young	44	15	26	24
UMN Old	44	47	45	42
UMN Young	44	31	43	30
Emory Old	44	29	40	41
Emory Young	44	34	32	31
USC Old	44	19	27	n/a
	All Groups & Ages	Alcohol-Exposed	Control	Contrast
Tested Total	689	219	263	207
Goal Total	880	308	308	264

1. Use existing data to develop a tiered or hierarchical approach to identification of affected cases.

Pertinent to this aim, we developed a decision tree model using 4 measures. We developed the model using data from CIFASD II and subsequently tested it on data collected in CIFASD III (see description of aims 2 and 3, immediately following). The objective of the study, as prescribed by Aim 1 was to improve identification of children who are affected by prenatal alcohol exposure. We were particularly interested in how well we could identify alcohol-exposed children who did not show facial dysmorphology that would otherwise identify them as having been alcohol exposed (i.e., the facial features seen in FAS). To develop the model, we evaluated over 1000 neurobehavioral and dysmorphology variables collected from 434 children (8-16y) with prenatal

Figure 1. Decision tree model

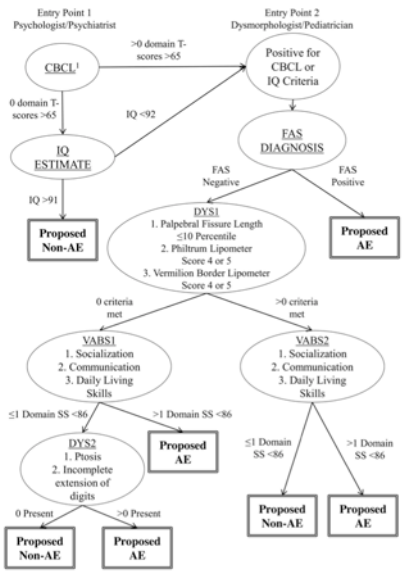


Table 2: Accuracy measures for the decision tree, developed using data from CIFASD I

Accuracy Measure	Entry Point 1	Entry Point 2
Overall Accuracy	*84.6%*	*80.1%*
Sensitivity	74.2%	79.2%
Specificity	89.9%	80.6%
Positive Predictive Value	78.6%	70.7%
Negative Predictive Value	87.4%	86.7%

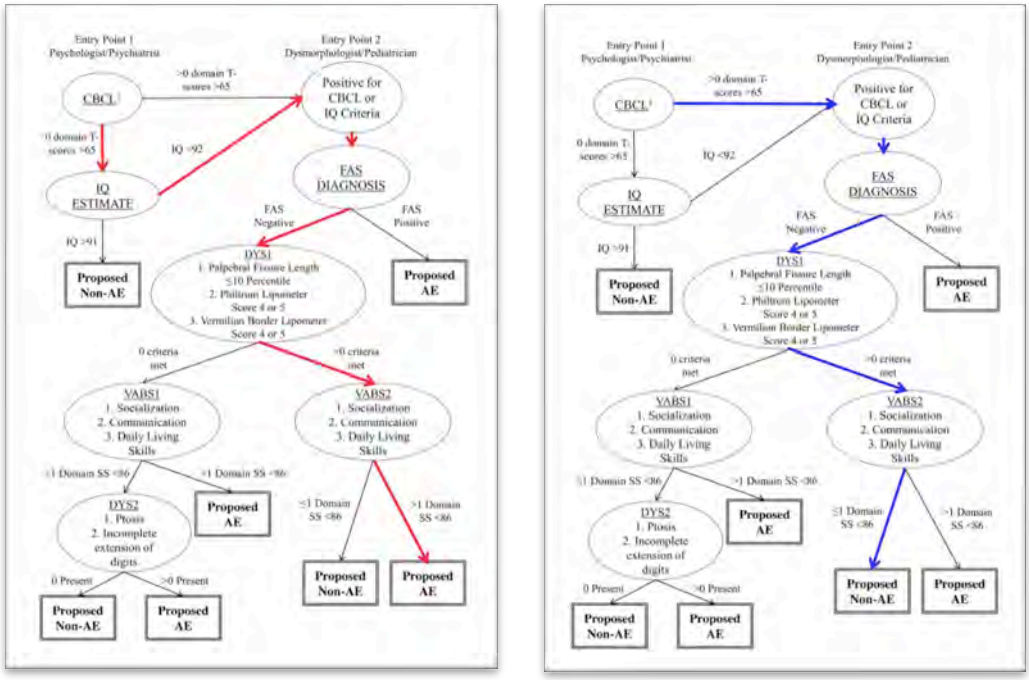
alcohol exposure, with and without FAS, and non-exposed controls, with and without other clinically-relevant behavioral or cognitive concerns. The discriminatory ability of each model step was tested with logistic regression. Classification accuracies and positive and negative predictive values were calculated. The final model consisted of variables from 4 measures (2 parent questionnaires, an IQ score, and a dysmorphology examination). Two entry points were modeled, based on clinical presentation to (1) a psychologist or other mental health provider, and (2) and dysmorphologist or pediatrician. Overall accuracy rates met or exceeded our goal of 80% overall accuracy. The decision tree is illustrated in **Figure 1** and the accuracy rates are presented in **Table 2**. Examples of two children follow.

Example 1: A child presents to a psychologist with a presenting complaint of behavior and school problems. Using the decision tree model, the psychologist administers the Child Behavior Checklist (CBCL). This parental report measure reveals that there are no areas that meet the cutoff of >65. The psychologist then administers an IQ test and the child has an IQ of 88, which meets the cutoff of <92. This child would then be referred to a dysmorphologist for examination. This exam reveals that while the child does not have FAS, he does have one of the features in the model (e.g., philtrum score of 4 or 5), prompting the administration of the VABS. This test reveals two elevated scores (e.g., communicaton *and* daily living skills). The combination of these data indicate that the child is likely to be alcohol-exposed based on the decision tree model. This path is shown on the left in **Figure 2**.

Example 2: A child presents to a psychologist with a presenting complaint of behavior and school problems and in this case has at least one elevated score on the CBCL. Using the decision tree model, the psychologist refers the child to a dysmorphologist for examination (no IQ test is needed). The dysmorphology exam reveals that, again, the child does not have FAS, and has no listed physical features features. VABS testing reveals 1 elevated score (e.g., communication). The combination of these data indicate that the child is not likely to be alcohol-exposed based on the decision tree model. This path is shown on the right in **Figure 2.**

While we originally proposed to include 3D facial imaging and MRI variables in the model, there were several reasons we elected not to in the final model, including sample size – the imaging subsample is smaller than the sample with neurobehavioral data – and clinical feasibility – we felt that many clinicians would not have access to the high level tools required for facial or brain imaging. Our model is parsimonious and meets our goal of creating a tool that is clinically efficient, inexpensive, and easy to use.

Figure 2. Decision tree model showing 2 possible paths.



2. Test the specificity and sensitivity of the model developed in Aim 1 in children ages 10-16.

AND

3. Test the utility of the model in younger children, ages 5-7.

We tested our model in two age groups (ages 10-16 [Aim 2] and 5-7 [Aim 3]. This independent validation sample consisted of 454 children from CIFASD III, including subjects with prenatal alcohol exposure, with and without FAS, and non-exposed controls, with and without other clinically-relevant behavioral or cognitive concerns. The model is the same as the figures, above. The accuracy rates are listed in **Table 3**. Based on our results, we concluded that our decision tree model is highly accurate and clinically efficient in distinguishing children affected by prenatal alcohol exposure from non-exposed controls, including those with other behavioral concerns or conditions. Improving identification of this population will streamline access to clinical services, including multidisciplinary evaluation and treatment.

Table 3. Accuracy ratings for the validation sample (CIFASD III)

	Entry Point 1		**Entry Point 2**	
Accuracy Measure	Child (5-7y)	Adolescent (10-16y)	Child (5-7y)	Adolescent (10-16y)
Overall Accuracy	*84.5%*	*84.7%*	*82.1%*	*79.5*
Sensitivity	70.7%	79.3%	63.8%	81.3%
Specificity	93.5%	87.6%	93.4%	78.3%
Positive Predictive Value	87.9%	77.4%	85.7%	71.4%
Negative Predictive Value	82.9%	88.7%	80.7%	86.2%

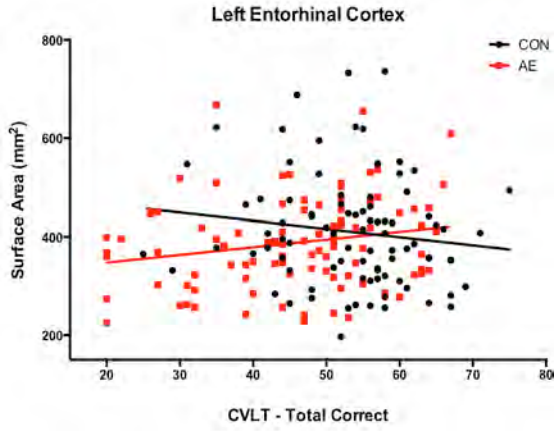
A paper describing the tree and results from the development and validation samples was submitted for publication in January.

In addition to the decision tree study, we examined the role of age directly in one additional study. Using data from CIFASD III, we tested whether alcohol-related deficits differed in the 2 target age groups and whether sex differences were noted. Results support alcohol-related deficits in all three domains tested (neuropsychological function, psychopathology, and behavior). However, there were no interactions with either age or sex. Although adolescents with prenatal alcohol exposure may perform more poorly than younger exposed children, the same was true for non-exposed children. Thus, these cross-sectional data indicate that the developmental trajectory for neuropsychological and behavioral performance is not altered by prenatal alcohol exposure but rather deficits are consistent across the two age groups tested. Similarly, observed sex differences on specific measures were consistent across the groups and do not support sexually dimorphic effects in these domains. This paper is in the final editing phase and we hope to submit it for publication by 4/1/16.

4. Targeted assessment of memory function.

We have completed 2 studies related to memory function using CIFASD III data. The first study examined the specificity of memory deficits in children and adolescents (ages 5-7 and 10-16) with prenatal alcohol exposure. We tested specificity by comparing multiple measures and including two comparison groups, a typical control (T-CON) group and a group with behavior concerns or conditions (B-CON). Results indicated that in general, the T-CON group had higher scores than the B-CON and AE group on all subtests, though profiles varied by group and age. Age differences were only observed in the B-CON group. This study was presented at INS this year and a paper is in the final editing phase. We hope to have it submitted by 4/1/16.

Figure 3. Relation between cortical surface area and CVLT-C performance



The second study examined neural correlates of memory function in children (ages 10-16y) with prenatal alcohol exposure and controls. An abstract on a portion of this study, examining surface area, was submitted to RSA. The results of that analysis showed differential relations between verbal learning measures from the CVLT-C and surface area of left entorhinal cortex and left and right parahippocampal cortices. Better CVLT-C performance related to increased cortical surface area in the AE group but decreased surface area in the CON group. These results are illustrated in **Figure 3**. Analyses are ongoing for additional neural measures (volume and cortical thickness); we hope to submit a paper by 6/15/16.

3. For this reporting period (April 1, 2015 - present), 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. Describe your project's **interrelation** with aims of the CIFASD consortium and other CIFASD 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 are 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 including facial imaging and brain imaging. The decision tree study, described above, is an excellent example of the collaboration between the dysmorphology and neurobehavior projects. The neural correlates study, also described above involves collaboration between the neurobehavioral and neuroimaging projects.

7. What do you **plan to do for the next reporting period** (March 1, 2016 - April 1, 2017) to accomplish your project's specific aims?

During the next funding year, we plan to complete data collection, continue to analyze data, and prepare the renewal application. With the studies described above and the publications in preparation (described above and listed below), we have addressed the aims of the current phase. During the next funding year, we will focus on examining the data for patterns and results that will help guide the application for renewed funding.

8. CIFASD Phase III ends in May of 2017. **What specific aims do you see your project addressing in the upcoming competitive renewal?**

There are several possible directions we can take in the competitive renewal. For example, we may examine the role of moderating factors in neurobehavioral outcomes. In the current period, we addressed the role of age and sex in the neurobehavioral effects and didn't find these to differentially affect the group. We could also examine the role of other exposures. Third, we could apply our decision model to new and diverse samples to test the reliability and validity of the model. Confirmation of the model in additional known samples will (hopefully) allow it to be deployed in samples with unknown exposures (i.e., adoption cases). A fourth idea is to combine our research on the neurobehavioral effects in FASD with new technology. For example, we could examine the possibility of developing a mobile application for deploying the decision tree model. Similarly, we could use electronic devices to actually administer measures that could be used to assess, diagnose, or classify subjects. Development of such applications would allow tools to be deployed in rural or underserved populations. For this type of study it would be beneficial to have a "new technologies" core in the renewal application. Finally, one area that could be studied is the effect of lower levels of exposure or exposure only prior to pregnancy recognition. Clinically these are the more common types of exposures. Understanding how lower levels of exposure affect brain/cognitive development requires additional study and would likely have significant clinical impact.

9. What do you believe the **hot areas of FASD research** to be?

Our work has demonstrated that there are still aspects of neurocognition that remain to be understood in FASD and that these aspects may be important in improving identification of affected children. The combination of dysmorphology and neuropsychological/neurobehavioral assessment is key to determining the range of effects. Moving the window of measurement to lower levels of exposure is a critical research need.

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

NIH Public Access Compliance	Citation
Complete	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. 2015 Dec;25(12):4761-71. PubMed PMID: 25092900; PubMed Central PMCID: PMC4635917.
Complete	Doyle LR, Mattson SN. Neurobehavioral Disorder Associated with Prenatal Alcohol Exposure (ND-PAE): Review of Evidence and Guidelines for Assessment. Curr Dev Disord Rep. 2015 Sep;2(3):175-186. PubMed PMID: 26509108; NIHMSID: NIHMS703603; PubMed Central PMCID: PMC4617308.

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

Goh, P.K., Doyle, L.R., Glass, L., Jones, K.L., Riley, E.P., Coles, C.D., Hoyme, H.E., Kable, J.A., May, P.A., Kalberg, W.O., Sowell, E.R., Wozniak, J.R., **Mattson**, S.N., and the CIFASD. A clinically useful decision tree to identify children affected by prenatal alcohol exposure [manuscript submitted for publication. CIFASD concept proposal submitted January 2015, CIFASD manuscript submission template completed January 2016]

Panczakiewicz, A.L., Glass, L., Coles, C.D., Jones, K.L., Kable, J.A., Sowell, E.R., Wozniak, J., Riley, E.P., **Mattson**, S.N., and the CIFASD. Neurobehavioral deficits do not vary by age or sex in children and adolescents with prenatal alcohol exposure [manuscript in preparation, hope to submit by 4/1/16; CIFASD concept proposal submitted January 2015]

Gross, L.A., Glass, L., Goh, P.K., Coles, C.D. Jones, K.L., Kable, J.A., Sowell, E.R., Wozniak, J.R., Riley, E.P., **Mattson**, S.N., and the CIFASD. Specificity of memory deficits in children and adolescents with prenatal alcohol exposure [poster presented at the International Neuropsychological Society meeting, Boston, February 2016; manuscript in preparation, hope to submit by 4/1/16; CIFASD concept proposal submitted January 2015].

Goh, P.K., Coles, C.D., Kable, J.A., Sowell, E.R., Wozniak, J.R., Riley, E.P. **Mattson**, S.N. and the CIFASD. Contributions of behavioral impairment to executive function deficits in children and adolescents with prenatal alcohol exposure [manuscript in preparation, hope to submit by 6/15/16; CIFASD concept proposal submitted January 2016]

Gross, L.A., Moore, E.M., Coles, C.D., Kable, J.A., Sowell, E.R., Wozniak, J.R., Riley, E.P. **Mattson**, S.N. and the CIFASD. Neural correlates of verbal memory in youth with histories of heavy prenatal alcohol exposure [abstract to be presented at the Research

Society on Alcoholism meeting, New Orleans, June 2016; manuscript in preparation, hope to submit by 6/15/16; CIFASD concept proposal submitted January 2015]

Cheney, D.C., Wozniak, J.R., Coles, C.D., Kable, J.A., Sowell, E.R., Riley, E.P. **Mattson**, S.N. and the CIFASD. Interaction of prenatal alcohol exposure and postnatal SES on general cognitive ability [poster to be presented at the Research Society on Alcoholism meeting, New Orleans, June 2016; manuscript in preparation, hope to submit by 8/1/16; CIFASD concept proposal submitted January 2016]

Doyle, L.R., Moore, E.M., Coles, C.D., Kable, J.A., Sowell, E.R., Wozniak, J.R., Riley, E.P. **Mattson**, S.N. and the CIFASD. Cognitive predictors of communication impairment in youth with histories of heavy prenatal alcohol exposure [abstract to be presented at the Research Society on Alcoholism meeting, New Orleans, June 2016; manuscript in preparation, hope to submit by 8/1/16; CIFASD concept proposal submitted January 2016]

12. **Poster Abstracts and Presentations**

Cheney, D.C., Wozniak, J.R., Coles, C.D., Kable, J.A., Sowell, E.R., Riley, E.P. **Mattson**, S.N. and the CIFASD (2016). Interaction of prenatal alcohol exposure and postnatal SES on general cognitive ability. To be presented at the Research Society on Alcoholism meeting, New Orleans, June 2016.

Gross, L.A., Moore, E.M., Coles, C.D., Kable, J.A., Sowell, E.R., Wozniak, J.R., Riley, E.P. **Mattson**, S.N. and the CIFASD (2016). Temporal cortex surface area predicts verbal memory in youth with histories of heavy prenatal alcohol exposure. To be presented at the Research Society on Alcoholism meeting, New Orleans, June 2016.

Doyle, L.R., Moore, E.M., Coles, C.D., Kable, J.A., Sowell, E.R., Wozniak, J.R., Riley, E.P. **Mattson**, S.N. and the CIFASD (2016). Cognitive predictors of communication impairment in youth with histories of heavy prenatal alcohol exposure. To be Presented at the Research Society on Alcoholism meeting, New Orleans, June 2016.

Gross, L.A., Glass, L., Goh, P.K., **Mattson**, S.N., and the CIFASD (2016). Specificity of memory deficits in children and adolescents with prenatal alcohol exposure. Presented at the International Neuropsychological Society meeting, Boston, February 2016.

Moore, E.M., Glass, L., Infante, M.A., Deweese, B.N., **Mattson**, S.N., Riley, E.P., and the CIFASD (2015). Developmental trajectory of spatial working memory performance is delayed and decelerated in children with heavy prenatal alcohol exposure. Presented at the Research Society on Alcoholism meeting, San Antonio, June 2015. doi: 10.1111/acer.12741, Published online 19 May 2015. http://onlinelibrary.wiley.com/doi/10.1111/acer.12741/abstract

Goh, P.K., Doyle, L.R., Glass, L., Jones, K.L., Riley, E.P., **Mattson**, S.N., & the CIFASD (2015). Developing a decision tree for clinical identification of children affected by prenatal alcohol exposure I: model development. Presented at the Research Society on Alcoholism meeting, San Antonio, June 2015. doi: 10.1111/acer.12741, Published online 19 May 2015. http://onlinelibrary.wiley.com/doi/10.1111/acer.12741/abstract

Doyle, L.R., Goh, P.K., Glass, L., Jones, K.L., Riley, E.P., **Mattson**, S.N., & the CIFASD (2015). Developing a decision tree for clinical identification of children affected by prenatal alcohol exposure II: model validation. Presented at the Research Society on Alcoholism meeting, San Antonio, June 2015. doi: 10.1111/acer.12741, Published online 19 May 2015. http://onlinelibrary.wiley.com/doi/10.1111/acer.12741/abstract

Mattson, S.N., Jones, K.L., Goh, P.K., Doyle, L.R., Riley E.P., & the CIFASD (2015). Decision tree for clinical identification of children affected by prenatal alcohol exposure. Presented at the Research Society on Alcoholism meeting, San Antonio, June 2015. doi: 10.1111/acer.12741, Published online 19 May 2015. http://onlinelibrary.wiley.com/doi/10.1111/acer.12741/abstract

Book Chapters

Doyle, L.R., Crocker, N.A., Fryer, S.L., and **Mattson**, S.N. (in press, 2016). Exposure to teratogens as a risk factor for psychopathology. In T.P Beauchaine and S.P. Hinshaw (Eds.), Child and Adolescent Psychopathology, 3rd editionHoboken, NJ: John Wiley & Sons, Inc.

Glass, L., and **Mattson**, S.N. (in press, 2016). Fetal Alcohol Spectrum Disorders: Academic and Psychosocial Outcomes. In C.A. Riccio and J.R. Sullivan (Eds.), Pediatric Neurotoxicology: Academic and Psychosocial Outcomes. New York, NY: Springer Publishing Company.

Graham, D.M., Glass, L., and **Mattson**, S.N. (2016). Teratogen exposure and externalizing behavior. In T.P Beauchaine and S.P. Hinshaw, (Eds.), Oxford Handbook of Externalizing Spectrum Disorders (pp. 416-439). New York, NY: Oxford University Press. Published online Sep 2014. doi:10.1093/oxfordhb/9780199324675.013.22

Principal Investigator(s): Kathleen K. Sulik and Scott E. Parnell
Institution(s): University of North Carolina
CIFASD Project Title: Craniofacial and CNS Pathology in a Mouse FASD Model
Grant Number: 5 U01 AA021651-04

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 (6/01/2015-5/31/16) our publications have included 1 full paper and 3 abstracts, with an additional 2 abstracts, a review and 2 book chapters having been submitted and 4 full papers currently in preparation.

Our major efforts during this funding period have been directed toward our Aim 3-related objectives, i.e. identification of environmental and genetic factors that influence alcohol's teratogenicity. The genetic pathway that we are currently focusing on is Shh; following up on previous publications by our group showing that the teratogenic effects of prenatal ethanol exposure are exacerbated by Sonic Hedgehog or Gli2 haploinsufficiency in mice [Kietzman et al, 2014; Fish et al, 2015 (RSA abst)] and that brain abnormalities resulting from Shh antagonist treatment (Lipinski et al, 2014) are consistent with those that alcohol causes. These findings have provided the foundation for a recently funded U54 subproject that is a collaborative effort between our laboratory and that of Kevin Williams at NCCU. With results based on alcohol-induced limb teratogenicity showing a role for Shh signaling modulation (Fish et al, 2015 abst; papers in prep), for the U54 study we are using the mouse limb as our model system and are conducting RNA seq analyses to identify genes that are both upstream and downstream of Shh whose modification may also influence alcohol's teratogenicity. This work has provided a foundation for a collaboration that we are pursuing with Johann Eberhart involving a comparable approach with the developing brain as the tissue of interest and with experiments conducted in both mice and fish. Additionally following up on Shh signaling interference as a mechanism underlying alcohol teratogenesis, and hypothesizing that alcohol acts by altering signal transduction through primary cilia, we have submitted an R01 proposal that explores the premise that defects within FASD represent a transient ciliopathy. And, focusing on the BAX gene, we are also further exploring the role that alterations in apoptosis-related genes may play in alcohol teratogenesis (Baker et al, abst submitted; paper in prep).

The environmental agents that we are focusing on are synthetic cannabinoid compounds; agents whose abuse is increasing and that are commonly concurrently used along with alcohol.

Our initial study has employed administration of CP-55,940 to mice on their 8th day of pregnancy (Gilbert et al, 2015). We have established a dose-response curve that shows neuro-teratogenicity of this compound at levels as low as 0.0625 mg/kg and are currently conducting alcohol co-exposure teratogenicity studies. Importantly, our data showing that CB1 receptors are present in the mouse embryo neuroepithelium on GD 8, coupled with recent work by Khaliullina et al.(2015) demonstrating that in *Drosophila*, circulating endogenous cannabinoids directly inhibit Shh pathway activation, along with our Shh/alcohol studies as noted above, are suggestive of interference with Shh signaling as a common teratogenic mechanism for cannabinoids and alcohol. Indeed, our on-going co-exposure studies indicate that these 2 teratogens act synergistically, with data in this regard having been submitted as a presentation for the 2016 CIFASD RSA symposium (Fish et al, 2016, abst submitted) and also accepted for an NIH cannabinoid meeting in March, 2016 (Fish et al.2016 abst). This research addresses novel issues of major public health concern.

Also, during this funding period we have unfortunately concluded that the dense surface modeling aspect of our Aim 1 brain/face analyses has not been productive. This is a primarily a result of attempting to expedite the MRI imaging to allow analyses of large numbers of animals. More specifically, placing more than one fetus in each MRI holder resulted in a significant amount of surface artifact, and, thus, the inability to confidently assess any facial shape differences between control and ethanol-exposed fetuses. With the GD 11.5 acute exposure time being novel for MRI analyses, we now plan to publish only the brain-related data for this group (Wieczorek et al, in prep).

3. For this reporting period (April 1, 2015 - present), is there one or more **Revision/Supplement** associated with this award for which reporting is required? Supplement U01 AA021651-01S1

Objectives and Goals: This supplement ended in June, 2015. It was directed toward promoting the health science training and career of Dr. Marcoita Gilbert and supported both research and mentoring/career development activities. The primary objective of the basic research was to make clinically significant discoveries regarding prenatal alcohol (ethanol) exposure-induced pathology involving the brain and face. This work naturally built on our CIFASD-supported basic research and addressed the need for a more complete understanding of the genetic and environmental factors that influence alcohol's teratogensis.

Dr. Gilbert's accomplishments/activities during the 2 months of funding through this supplement included participation in a Gordon conference on Cannabinoid Function in the CNS that was held in Lucca, Italy in May, 2015. Additionally she began preparation of a full paper regarding the teratogenicity of the synthetic cannabinoid, CP-55,940. Her research findings have provided the foundation for our laboratory's continuing work examining the teratogenicity associated with co-exposure to alcohol and synthetic cannabinoids as noted above.

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

Both Drs. Wieczorek and Gilbert continued their postdoctoral training with partial funding from this grant. Dr. Wieczorek worked primarily on analyses of the data from the Aim 1 studies and is currently finalizing a paper regarding this work for publication. While her postdoctoral tenure in our lab ended in December, 2015, she continues to work with us on a part time basis while attending nursing school at UNC. Dr. Gilbert's postdoctoral tenure in our laboratory ended in October of 2015. Prior to leaving the lab, she submitted her manuscript on CP-55,940 teratogenicity for publication. 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?

Dr. Sulik presented the results of her U01-supported studies at the Gillberg Neuropsychiatry Center in Gothenburg, Sweden in August, 2015. Additionally, as the recipient of the NIAAA 2015 Keller Award, she presented her laboratory's research at NIH in November of 2015.

Dr. Gilbert presented a talk entitled "Exploring the Teratogenicity of Early Gestational Synthetic Cannabinoid Exposure in Mice - Implications for Education and Prevention in Humans", at a Gordon conference in Lucca Italy in May, 2015.

Dr. Parnell presented a talk for the UNC Bowles Center on Ciliopathies on April 8, 2015 and gave a talk at the American Psychiatric Association meeting in Toronto, Canada in May, 2015.

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

Our studies assessing genetically modified mice and their susceptibility to alcohol teratogenesis complement and extend the work of Drs. Johann Eberhart and Tatiana Faroud. Our MRI-based studies complement those of Dr. Sowell and Dr. Wozniak.

7. What do you **plan to do for the next reporting period** (March 1, 2016 - April 1, 2017) to accomplish your project's specific aims?

Present RSA abstracts in June, 2015.

Submit an R01 application in June to support additional studies of cannabinoid/alcohol co-teratogenicity in a mouse model.

Publish a manuscript reporting GD11.5 MRI-based brain data.

Publish a manuscript reporting cannabinoid/alcohol interactions.

Publish a manuscript reporting that deletion of Bax can attenuate ethanol's teratogenesis during gastrulation.

Publish a manuscript reporting that activating the Shh pathway can induce polydactyly and another reporting that removing one copy of Shh increases limbs' susceptibility to ethanol's teratogenesis.

With Dr. Sulik retiring at the end of June, 2016, Dr. Scott Parnell will serve as the sole PI for the remainder of the funding period. During this time he will focus on continuing to identify genetic and environmental modifiers of ethanol's teratogenesis that contribute to altering an individual's susceptibility to early gestational ethanol exposure.

8. CIFASD Phase III ends in May of 2017. **What specific aims do you see your project addressing in the upcoming competitive renewal?**

Aim 1. To employ a non-biased approach (high-throughput transcriptome sequencing; RNA-Seq) to identify genetic modifiers of ethanol teratogenesis. Previous human-based analyses as well as work with animal models (esp. work conducted in our laboratories with mice and fish) have shown that differing sensitivities to ethanol-induced teratogenesis are associated with genetic background differences. Employing state of the art high-throughput transcriptome sequencing (RNA-Seq) as a non-biased approach that has proven effective in mammalian systems, the proposed studies are directed toward comprehensively identifying additional genetic modifiers of ethanol teratogenesis. For this Aim, initial emphasis will be placed on RNA-seq analyses of developing brain tissue from gastrulation and neurulation-stage mouse embryos, with comparisons being made between comparably staged alcohol-exposed and control samples. Based on developmental biological knowledge and feasibility, a subset of the identified genes will then be examined in fish in order to explore their influence on sensitivity to

ethanol teratogenesis. Finally, those genes whose impact is the greatest in the fish model, will be examined in mice with respect to their influence on ethanol-induced dysmorphology and neurobehavioral alterations. Genes whose modification confer the greatest sensitivity or resistance to these end-points will serve as candidates for study (with Dr. Foroud) in human populations.

Aim 2. To characterize suppressors of ethanol teratogenesis. Through our genetic approach, we have identified a set of mutants that greatly enhance the teratogenicity of low doses of ethanol. Using our understanding of the genetic pathway we were able to identify genetic and chemical suppressors of the ethanol-induced phenotype. While it is possible to identify suppressors based on such candidate approaches, these approaches are necessarily biased and relatively slow. It is our goal to perform an unbiased high throughput screen to identify a large number of suppressors. Several of the mutants that we have identified are haploinsufficient in the presence, but not absence, of ethanol, allowing for a novel method of screening for suppressors of gene-ethanol interactions. Additionally, slightly higher doses of ethanol result in stage specific defects within wild-type embryos, particularly when exposures are given during gastrulation. Using both forward genetic approaches and chemical library screening, we will A) Identify suppressors of gene-ethanol interactions and B) Characterize stage specific suppressors of ethanol teratogenicity. Once identified in zebrafish, we will prioritize further analyses, including verification in mouse, based upon 1) evidence for homologous interactions in human (with Dr. Foroud) and 2) feasibility and function during development.

9. What do you believe the **hot areas of FASD research** to be?

- Examination of genetic, environmental, and prenatal alcohol exposure interactions; i.e. identification of susceptibility and resistance factors
- Research directed toward improving/expanding FASD diagnosis, coordinated with research translation efforts.
- Globally-directed epidemiology research informed by an understanding of racial variability in presentation of symptoms

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

NIH Public Access Compliance	Citation
In process at NIHMS	Gilbert MT, Sulik KK, Fish EW, Baker LK, Dehart DB, Parnell SE. Dose-dependent teratogenicity of the synthetic cannabinoid CP-55,940 in mice. Neurotoxicol Teratol. 2015 Dec 18;PubMed PMID: 26708672; NIHMSID: NIHMS748198. [Epub ahead of print]

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

Sulik, KK, Normal and Abnormal Oro-facial Embryogenesis. Craniofacial Syndromes. G Dalben (ed), Bentham Science (submitted, 2015)

Sulik, KK, The embryonic origins of FASD: Animal-based studies of prenatal alcohol exposure and abnormal brain development, In: Ethical and Legal Perspectives in Fetal Alcohol spectrum Disorders: Foundational Issues, Springer (submitted, 2015)

Eberhart JK and Parnell SE. The Genetics of Fetal Alcohol Spectrum Disorders (FASD). Alc Clin Exp Res (submitted, 2016).

Baker LK, Fish EW, Mendoza-Romero HN, Parnell SE. The pro-apoptotic gene, Bax, mediates vulnerability to the craniofacial and brain abnormalities of FAS (In Prep)

Wieczorek L, Sulik KK, Suttie M, Lipinski M, Fish EW, Chapman W, Cofer GP, O'Leary-Moore SK, Budin F, Hammond P, and Parnell, SE. High throughput brain segmentation reveals prenatal ethanol exposure-induced dysmorphology in fetal mice (In Prep)

Fish EW, Sulik KK, Williams KP, Parnell SE. Vulnerability to prenatal alcohol-induced limb defects: Haploinsufficiency in sonic hedgehog pathway genes (In Prep)

Fish EW, Parnell SE, Sulik KK, Williams KP. Preaxial polydactyly following early gestational ethanol exposure to the smoothened agonist SAG in C57Bl6J Mice (In Prep)

12. **Poster Abstracts and Presentations**

Fish EW, Sulik KK, Williams KP, Parnell SE. Vulnerability To Prenatal Alcohol-Induced Limb Defects: Haploinsufficiency In Sonic Hedgehog Pathway Genes. Alcohol Clin Exper Res 39(S1):227A, 2015

Gilbert, M. et al, Exploring the Teratogenicity of Early Gestational Synthetic Cannabinoid Exposure in Mice - Implications for Education and Prevention in Humans, Gordon Research Conference on Cannabinoid function in the CNS, Lucca Italy, May 2015

Fish EW, Gilbert MT, Sulik KK, Parnell SE. Ethanol and the synthetic cannabinoid, CP-55,940, exhibit synergistic teratogenicity in a mouse model. Marijuana and Cannabinoids: A Neuroscience Research Summit, NIH, Bethesda, MD, March 2016

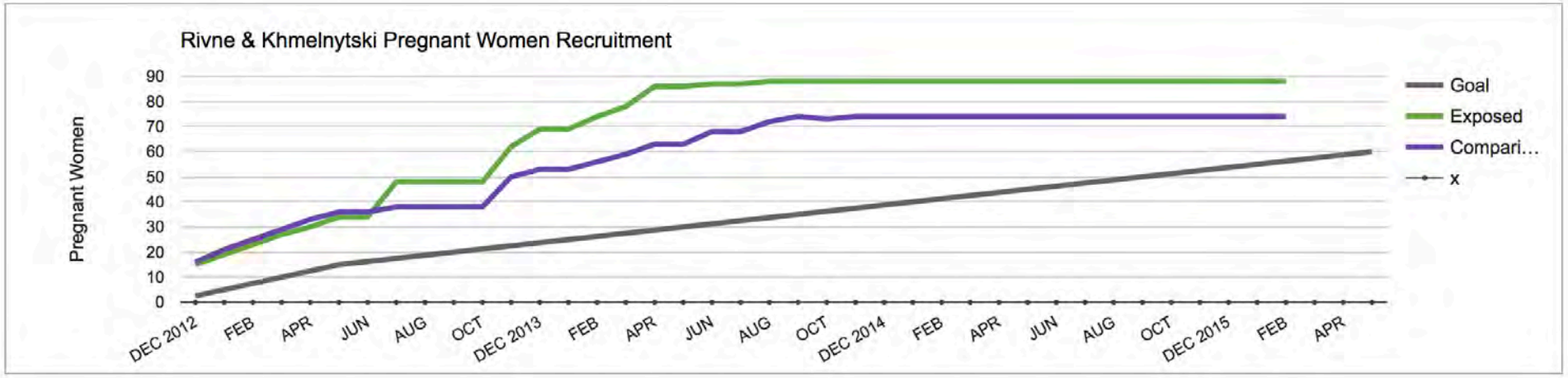
E.W. Fish, M.T. Gilbert, K.K. Sulik, S.E. Parnell. Ethanol And The Synthetic Cannabinoid, CP-55,940, Are Synergistically Teratogenic In A Mouse Model (submitted for 2016 RSA)

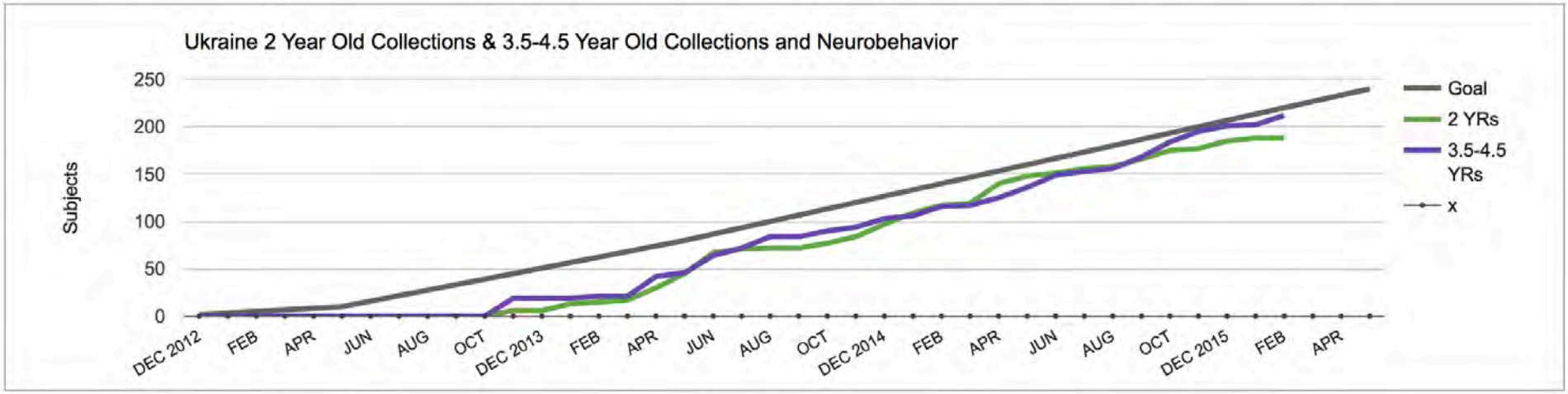
L.K. Baker, E.W. Fish, S.E. Parnell. Knocking Out The Pro-Apoptotic Bax Alters Susceptibility To The Characteristic Craniofacial Abnormalities Of FAS (submitted for 2016 RSA)

Google Graph Progress as of February 29th, 2016

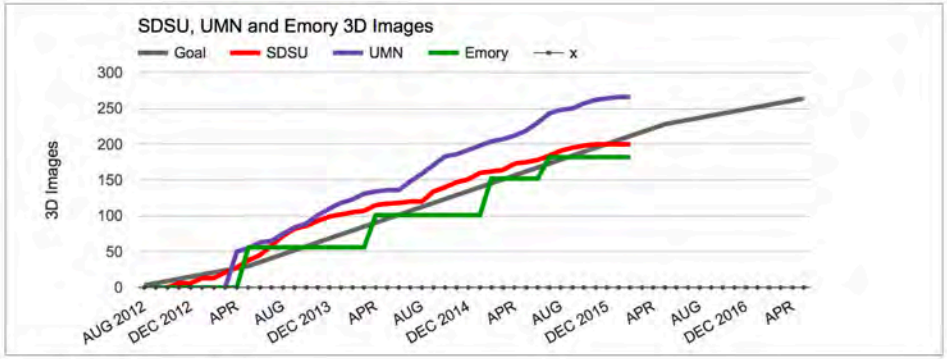
Ukraine Progress

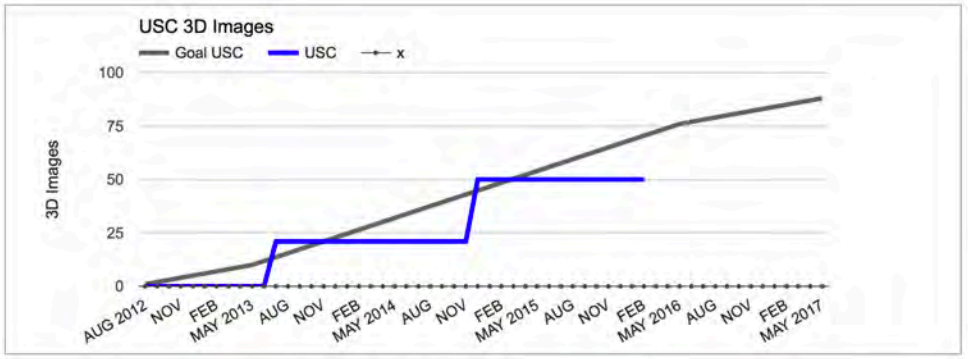
X in the keys that follow is to allow for a line to run along the x-axis indicating a hash mark (dot) per month.

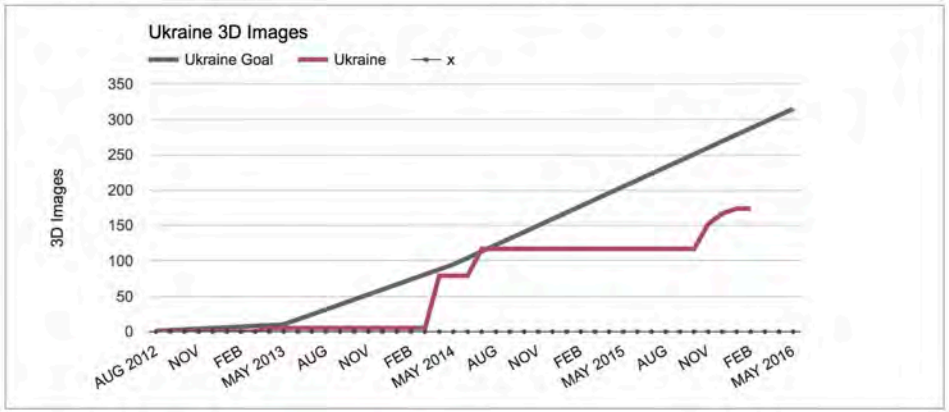


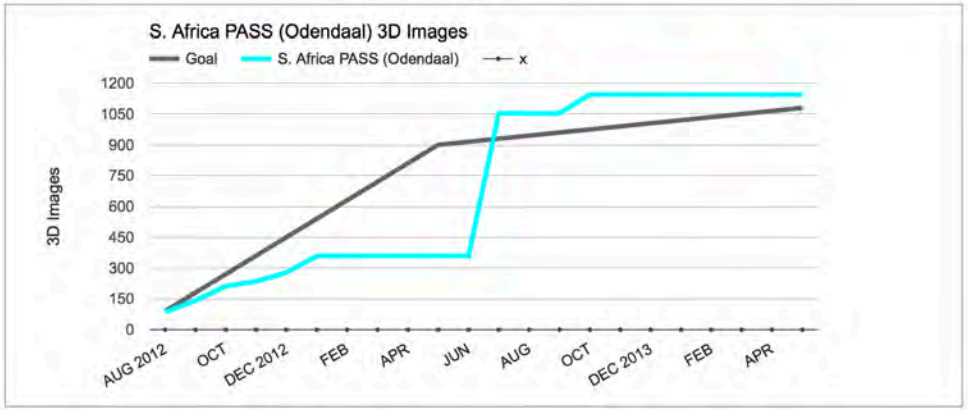


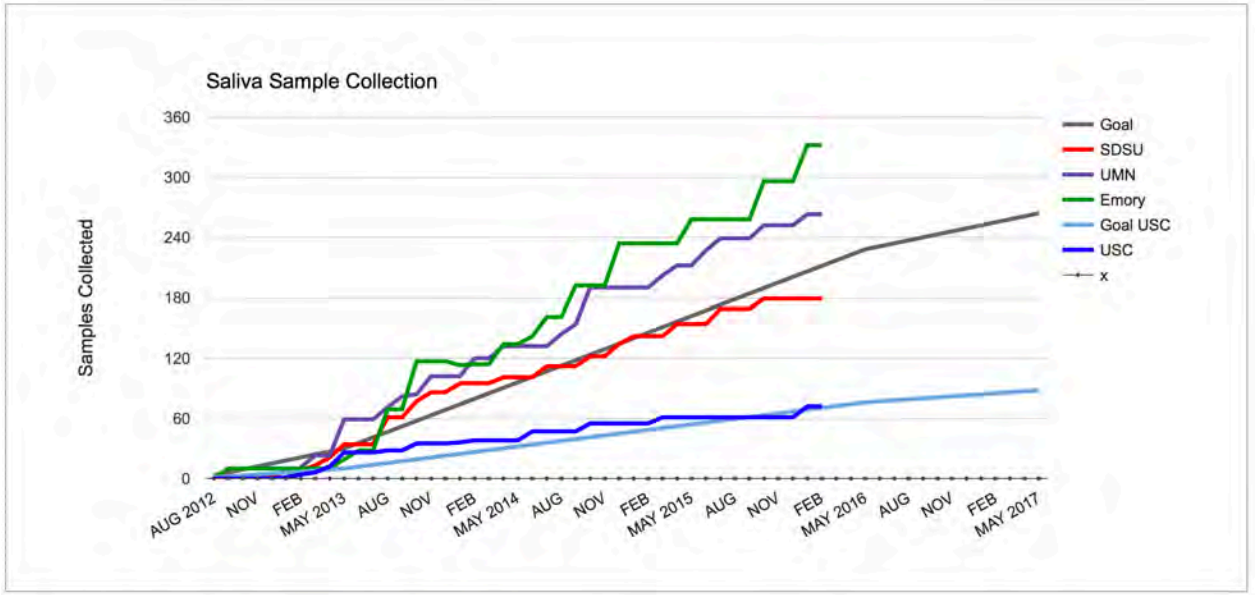
3D Images & Saliva Sample Collection [294 3D Images at the South Africa (Jacobsons' site) were collected in Phase III.]



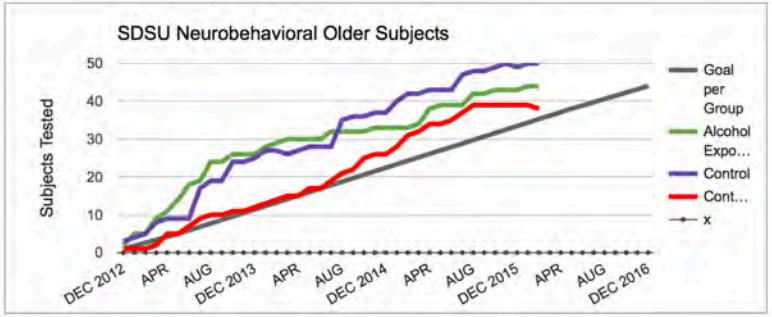


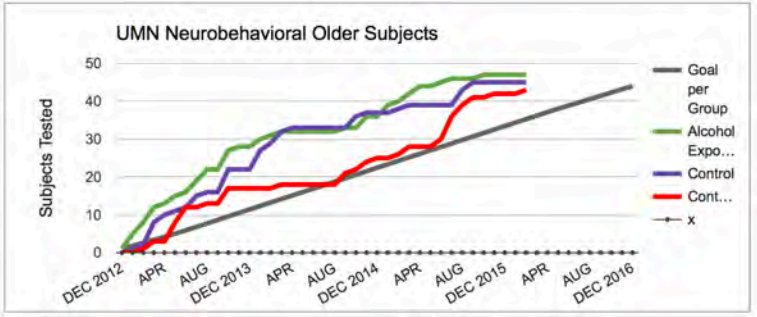


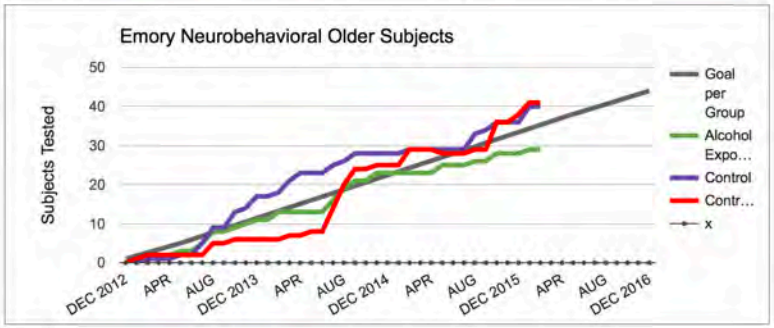


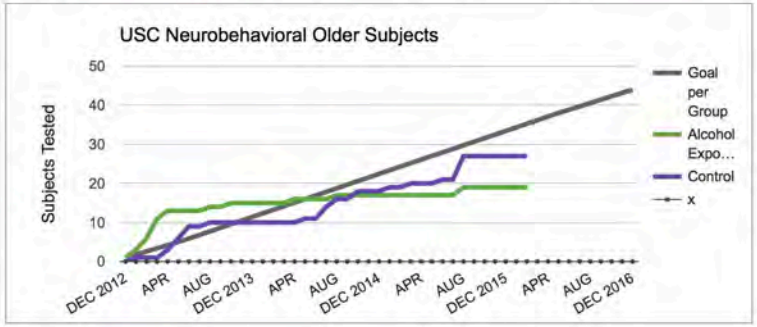


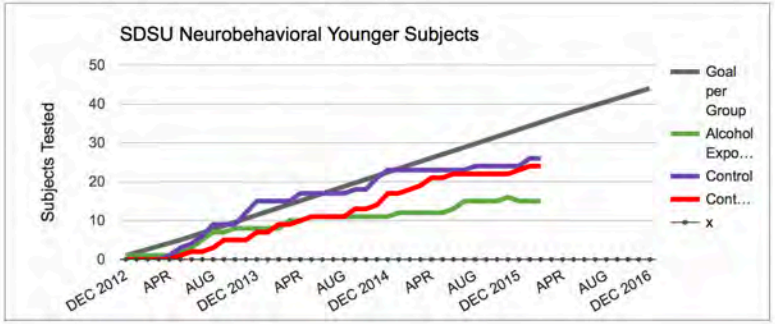
Neurobehavioral Subjects

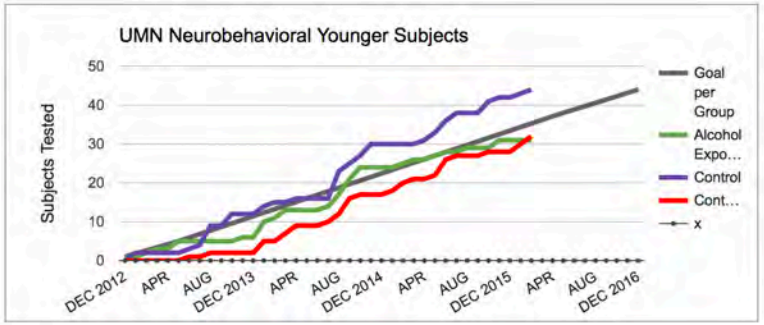


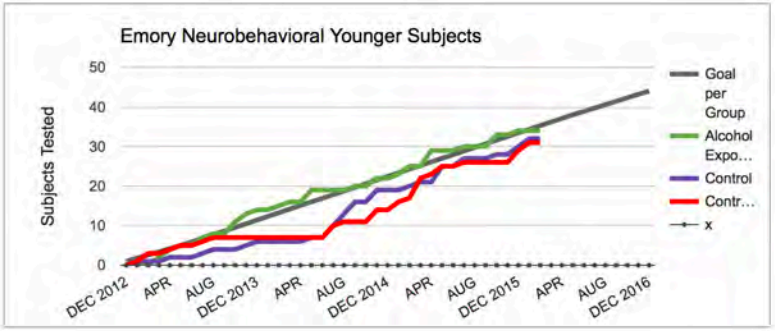




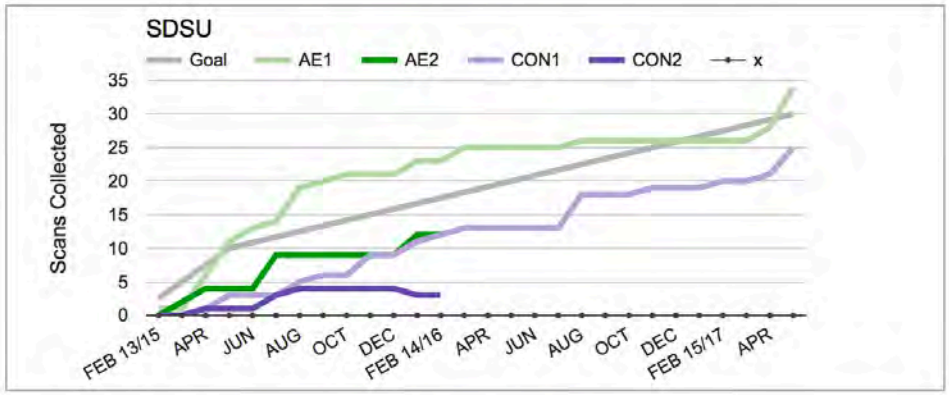


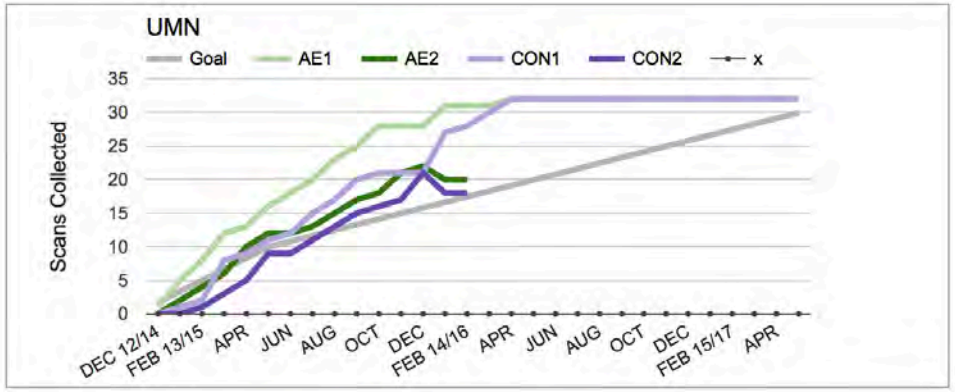


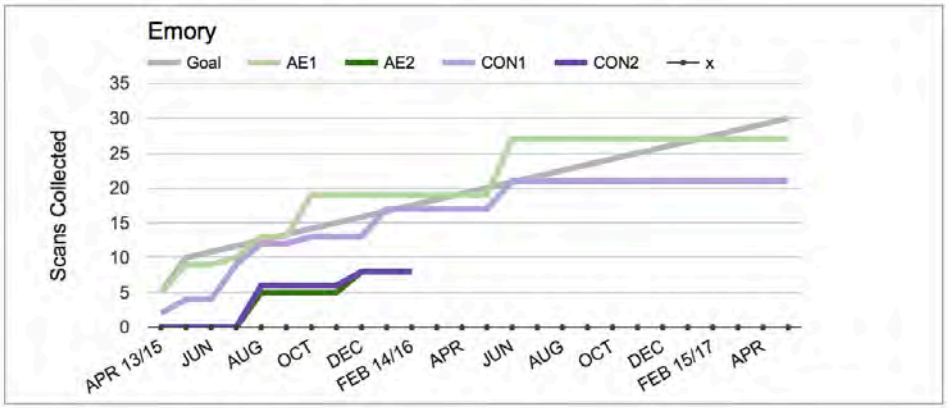


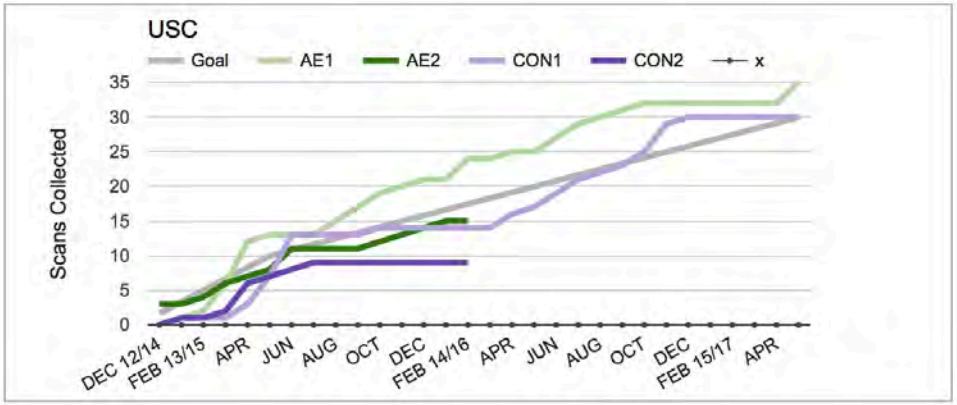


Neuroimaging Subjects
AE1 = Alcohol exposed subjects 1st scans. AE2 = Alcohol exposed subjects 2nd scans. CON = Control.









Note regarding reduction at some sites: Per project personnel, the numbers entered each month are from imaging uploads. Recently, the project double checked with all sites via email to PIs to confirm the numbers and fixed them accordingly.

Percentages to Overall Phase III Goals

Neuroimaging

Percent to Overall Goals	SDSU	UMN	Emory	USC
Time 1 AE	113%	107%	90%	117%
Time 1 CON	83%	107%	70%	100%
Time 2 AE	40%	67%	27%	50%
Time 2 CON	10%	60%	27%	30%

Ukraine

Ukraine Aims	% to Goal
Recruitment Exposed	147%
Recruitment Comparison	123%
2 YR Old Collections	78%
3.5-4.5 YR Neurobehavior & Collections	88%

Time into 5-Year Segment:	
3	Years
9	Months
	#s thru FEB 2016

Neurobehavioral

Old Percent to Goal	SDSU	UMN	Emory	USC
Alcohol-Exposed	100%	107%	66%	43%
Control	114%	102%	91%	61%
Contrast	86%	98%	93%	n/a
Young Percent to Goal	**SDSU**	**UMN**	**Emory**	
Alcohol-Exposed	34%	70%	77%	
Control	59%	100%	73%	
Contrast	55%	73%	70%	

3D Imaging & Saliva

3D Images	% to Goal
SDSU	76%
UMN	101%
Emory	69%
USC	57%
Ukraine	55%
S. Africa PASS (Odendaal)	106%
S. Africa (Jacobsons)	134%

Saliva Samples	% to Goal
SDSU	68%
UMN	100%
Emory	126%
USC	82%

Total NB Tested	
SDSU	197
UMN	242
Emory	207
USC	46
TOTAL	692

CIFASD Publications Citing Current CIFASD Grants

Not previously reported; Sources - CIFASD.org PubMed list and progress reports.

Charness ME, Riley EP, and Sowell ER. Drinking during pregnancy and the developing brain: Is any amount safe? *Trends in Cognitive Sciences,* 2016 Feb;20(2):80-82. NIHMSID: NIHMS763563

Coles CD, Kable JA, Keen CL, Jones KL, Wertelecki W, Granovska IV, Pashtepa AO, and Chambers CD; CIFASD. Dose and timing of prenatal alcohol exposure and maternal nutritional supplements: Developmental effects on 6-month-old infants. *Maternal and Child Health Journal*, 2015 Dec;19(12):2605-2614. PMCID:PMC4644455

Doyle LR, and Mattson SN. Neurobehavioral Disorder Associated with Prenatal Alcohol Exposure (ND-PAE): Review of evidence and guidelines for assessment. *Current Developmental Disorders Reports*, 2015 Sep;2(3):175-186. PMCID:PMC4617308

Gautam P, Lebel C, Narr KL, Mattson SN, May PA, Adnams CM, Riley EP, Jones KL, Kan EC, and Sowell ER. Volume changes and brain-behavior relationships in white matter and subcortical gray matter in children with prenatal alcohol exposure. *Human Brain Mapping*, 2015 Jun;36(6):2318-2329. PMCID:PMC4631525

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*, 2015 Dec;25(12):4761-4771. PMCID:PMC4635917

Gilbert MT, Sulik KK, Fish EW, Baker LK, Dehart DB, and Parnell SE. Dose-dependent teratogenicity of the synthetic cannabinoid CP-55,940 in mice. *Neurotoxicology and Teratology*, 2015 Dec 18. [Epub ahead of print] NIHMSID: NIHMS748198

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*, 2015 Sep 1;148:45-50. PMCID:PMC4408220

Infante MA, Moore EM, Bischoff-Grethe A, Migliorini R, Mattson SN, and Riley EP. Atypical cortical gyrification in adolescents with histories of heavy prenatal alcohol exposure. *Brain Research*, 2015 Oct 22;1624:446-454. PMCID:PMC4630133

Kable JA, Coles CD, Keen CL, Uriu-Adams JY, Jones KL, Yevtushok L, Kulikovsky Y, Wertelecki W, Pedersen TL, and Chambers CD; CIFASD. The impact of micronutrient supplementation in alcohol-exposed pregnancies on information processing skills in Ukrainian infants. *Alcohol*, 2015 Nov;49(7):647-656. PMCID:PMC4636447

Lee HS, Jones KL, Lee HK, and Chambers CD. Fetal alcohol spectrum disorders: Clinical phenotype among a high-risk group of children and adolescents in Korea. *American Journal Of Medical Genetics, Part A*, 2016 Jan;170(1):19-23. PMCID:PMC4715586

Lovely CB, Nobles RD, and Eberhart JK. Developmental age strengthens barriers to ethanol accumulation in zebrafish. *Alcohol*, 2014 Sep;48(6):595-602. PMCID:PMC4163099

Migliorini R, Moore EM, Glass L, Infante MA, Tapert SF, Jones KL, Mattson SN, and Riley EP. Anterior cingulate cortex surface area relates to behavioral inhibition in adolescents with and without heavy prenatal alcohol exposure. *Behavioural Brain Research,* 2015 Oct 1;292:26-35. PMCID:PMC4558293

Moore EM, and Riley EP. What happens when children with fetal alcohol spectrum disorders become adults? *Current Developmental Disorders Reports*, 2015 Sep;2(3):219-227. PMCID:PMC4629517

Murawski NJ, Moore EM, Thomas JD, and Riley EP. Advances in diagnosis and treatment of fetal alcohol spectrum disorders: From animal models to human studies. *Alcohol Research: Current Reviews*. 2015;37(1):97-108. Review. PMCID:PMC4476607

Tesche CD, Kodituwakku PW, Garcia CM, and Houck JM. Sex-related differences in auditory processing in adolescents with fetal alcohol spectrum disorder: A magnetoencephalographic study. *NeuroImage. Clinical,* 2015;7:571-587. PMCID:PMC4459049

Vakhtin AA, Kodituwakku PW, Garcia CM, and Tesche CD. Aberrant development of post-movement beta rebound in adolescents and young adults with fetal alcohol spectrum disorders. *NeuroImage. Clinical,* 2015;9:392-400. PMCID:PMC4589820

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 May;49(3):207-217. PMCID:PMC4414725

CIFASD Publications Citing Previous CIFASD Grants

Birch SM, Lenox MW, Kornegay JN, Shen L, Ai H, Ren X, Goodlett CR, Cudd TA, and Washburn SE. Computed tomography assessment of peripubertal craniofacial morphology in a sheep model of binge alcohol drinking in the first trimester. *Alcohol*, 2015 Nov;49(7):675-689. PMCID:PMC4636442

Cheng DT, Jacobson SW, Jacobson JL, Molteno CD, Stanton ME, and Desmond JE. Eyeblink classical conditioning in alcoholism and fetal alcohol spectrum disorders. *Front Psychiatry*, 2015;6:155. PMCID:PMC4629452

du Plessis L, Jacobson SW, Molteno CD, Robertson FC, Peterson BS, Jacobson JL, and Meintjes EM. Neural correlates of cerebellar-mediated timing during finger tapping in children with fetal alcohol spectrum disorders. *NeuroImage. Clinical*, 2015;7:562-570. PMCID:PMC4377649

Fan J, Meintjes EM, Molteno CD, Spottiswoode BS, Dodge NC, Alhamud AA, Stanton ME, Peterson BS, Jacobson JL, and Jacobson SW. White matter integrity of the cerebellar peduncles as a mediator of effects of prenatal alcohol exposure on eyeblink conditioning. *Human Brain Mapping*, 2015 Jul;36(7):2470-2482. PMCID:PMC4478219

Lewis CE, Thomas KG, Dodge NC, Molteno CD, Meintjes EM, Jacobson JL, and Jacobson SW. Verbal learning and memory impairment in children with fetal alcohol spectrum disorders. Alcoholism: Clinical and Experimental Research, 2015 Apr;39(4):724-732. PMCID:PMC4384160

Lindinger NM, Malcolm-Smith S, Dodge NC, Molteno CD, Thomas KG, Meintjes EM, Jacobson JL, and Jacobson SW. Theory of mind in children with fetal alcohol spectrum disorders. *Alcoholism: Clinical and Experimental Research*, 2016 Feb;40(2):367-376. PMCID:PMC4743533

Woods KJ, Meintjes EM, Molteno CD, Jacobson SW, and Jacobson JL. Parietal dysfunction during number processing in children with fetal alcohol spectrum disorders. *NeuroImage. Clinical,* 2015;8:594-605. PMCID:PMC4506983

Zhou FC. Dissecting FASD through the global transcriptome. *Alcoholism: Clinical and Experimental Research*, 2015 Mar;39(3):408-12. PMCID:PMC4348334