B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

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

Aim 2. Facilitate communication among the various projects and the dissemination of results. Communication is maintained with the CIFASD website, scheduled monthly conference calls, biannual meetings, and formal progress reports. The AdminC oversees the interaction of NOFAS with research components, allowing for the timely and accurate dissemination of scientific knowledge. It maintains an archive of publications, presentations, progress reports, and evaluations.

Aim 3. Assist with data management strategies. The AdminC provides assistance with data collection to ensure that data from the projects are uploaded into the Central Repository in a timely fashion, and accessible to all CIFASD PIs and approved outside investigators. The AdminC works closely with the Informatics component to enhance the online interactive capacity of CIFASD.

Aim 4. Provide annual evaluations of progress. The AdminC assists the SAB and NIAAA in the evaluation of projects, integration, and future directions of CIFASD components. In conjunction with the SAB and the Steering Committee, it establishes annual priorities and manages issues related to the allocation of resources.

Aim 5. Provide eHealth assistance. To make recruitment and outreach more accessible, the AdminC assists projects with electronic health strategies (eHealth) related to their research. There is little doubt that eHealth is becoming an important modality in healthcare, and CIFASD is ideally positioned to bring eHealth to the field of FASD research.

By its very nature, the AdminC interrelates with each project and committee within the CIFASD consortium.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

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B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

In April 2018, Dr. Riley was the moderator of a “Let’s Talk” pre-conference session on “Brain Sciences – Imaging & Neuropsychology” at the 8th International FASD Adolescents and Adults in held in Vancouver, Canada. In June 2018, he was the moderator and discussant of the CIFASD-focused symposium at RSA in San Diego, CA. In September 2018, Dr. Riley traveled to Kyoto, Japan to serve as the chair and discussant of the CIFASD-focused symposium at ISBRA. Later in September, he attended the EUFASD meeting in Berlin, Germany serving as the co-chair of the plenary session on prevention, the discussant in an audience involved engagement on international collaborations toward prevention, and as a plenary speaker in a separate session providing an update on the current research of CIFASD. Dr. Charness, in additional to assistance in organizing the CIFASD-themed symposia and plenary session at various meetings, also promotes CIFASD through talks at scientific meetings. Both he and his mentored students presented at the Vancouver 2018 meeting.

The Lancet Neurology invited review article on FASD being authored by Drs. Riley, Charness and Wozniak is in its third revision. Importantly, 14 new publications citing CIFASD funding have been published since the San Diego June 2018 meeting. Throughout this consortium’s tenure, the total number of publications citing CIFASD funding is now over 250.

NOFAS, who in the previous iteration of CIFASD was the Educational Component, received some funds for use through May 2018 through an approved carryover request submitted to NIAAA by the AdminC. With that support, they were able to disseminate CIFASD research findings during various forums where representatives of NOFAS, namely Tom Donaldson and Kathy Mitchell, were making presentations to broad audiences (ranging from caregivers of individuals with FASD to medical professionals to policy makers),

conducting/participating in interviews and providing briefings in meetings with legislative staff members. They also promoted recent CIFASD research, including links to the participant registries, via social media formats such as Twitter. Kathy Mitchell was able to assist Christie Petrenko with the arrangements for a focus group with families impacted by FASD, critical to Christie's CIFASD U01 research aims. Through their outreach, education about CIFASD and its research reaches a larger and more diverse audience resulting in improved opportunities and resources for individuals with FASD and their community of support.

The AdminC continues to maintain the consortium's website highlighting current research findings and accolades of CIFASD investigators on its homepage. Impactful news items during this reporting period included a two-part PBS NewsHour program on FASD where Jeff Wozniak was interviewed and his imaging work was featured. Views of the videos are not tallied on the PBS website; however, when accessed via YouTube, Part 1 has been viewed nearly 6,500 times. Furthermore, PBS NewsHour started a discussion forum due to the chatter about the pieces in their own newsroom. The rollout of the CIFASD-funded DiG FASD online enrollment platform was advertised in October 2018 and has also been showcased through the many social media tools and outlets utilized by NOFAS.

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Dr. Riley will be leading a search for another researcher to come aboard as a member of the CIFASD Science Advisory Board. He will also be working on restructuring the Data Access Committee as Bill Barnett and Helen Yezerets, members of the former Informatics Core, have moved on from CIFASD. Email communications and teleconferences with the other committee members will occur to ensure external data use requests are processed in a timely manner. Dr. Riley will also focus on the quest for CIFASD projects to have the resources and expertise needed to integrate and utilize mobile technologies and eHealth platforms into their projects to expedite the completion of the consortium's aims. Dr. Charness will be working with CIFASD investigators to assemble and submit a proposal for a CIFASD-themed symposium for presentation consideration at the September 2019 ESBRA Congress to be held in Lille, France.

As the current CIFASD UH2 projects (Blanchard/Mooney and Hashimoto-Torii/Torii) will be wrapping up their second and final project periods in May 2019, the AdminC will work with the NIAAA advisors to assist in any way needed with the recruitment of new UH2 proposals to be considered for funding starting in June of 2020.

B.2 What was accomplished under these goals?

1) Major Activities. The Collaborative Initiative on FASD (CIFASD) is a multisite, multidisciplinary consortium addressing the issue of fetal alcohol spectrum disorders by utilizing both basic and clinical research paradigms. The Administrative Core (AdminC) has the responsibility of providing the necessary administrative and scientific leadership and oversight to achieve the aims of all consortium components.

2) Specific Objectives. The Specific Objectives of the AdminC are stated in the Specific Aims (B.1).

3) Significant Results. As the AdminC is not responsible for conducting any research studies itself, its progress is reported in the next section, Key Outcomes and Other Achievements.

4) Key Outcomes and Other Achievements. Throughout the spring of 2018, the AdminC successfully coordinated, moderated and convened the monthly teleconference and WebEx meetings whose agendas, also set by the AdminC, primarily included the remainder of project overviews and updates by consortium PIs to wrap up the first year of CIFASD4. A face-to-face meeting was held on Friday, June 15, 2018 in San Diego, CA from the afternoon to early evening prior to the RSA meeting. The agenda included discussions of common study elements, project interactions, assessment of project needs and resource sharing and the interjection of common study elements into specific projects. An overview of the newest NIAAA data sharing policy for human subject research grants was provided and a group was formed, spearheaded by the team at IUPUI, to complete a verification of common consent language across all consortium projects to avoid any complications with future external data sharing. Other agenda items included presentations on plans for energizing cross-center/consortium interactions, brainstorming the framework for future CIFASD-focused plenaries/symposia and brief five-minute project progress updates by each PI. The early fall 2018 monthly meetings continued the discussions that began in San Diego concerning shared consortium protocols and data, integrations with other alcohol center grants and progress tracking for CIFASD4. Focus was placed on the action items required by all projects to accomplish these charges. The AdminC assisted with the protocols, reminders, collection and organization of the requested information. In November, Terry Deak, the Center Director of the Developmental Exposure Alcohol Research Center (DEARC) at Binghamton University, joined the first half of the monthly meeting to give an overview of the research being conducted by DEARC investigators and to open up the door to discussions about future collaborations between investigators of the two consortia. John Hannigan, a member of the SAB, volunteered to oversee additional ideas for cross-consortium planning. Ideas included a joint symposium and a social event at an upcoming conference. The December 2018 and January 2019 meetings served as mid-year progress checkpoints with each project giving an approximately five-minute update with a couple of slides via WebEx.

Beyond these monthly meetings, Dr. Riley and other AdminC personnel were involved in small group task-focused teleconferences to discuss and move forward the data sharing capabilities of the Central Repository for all phases of the consortium. Data from the first three phases of CIFASD is now ready to be released to external investigators, pending approval for each request through the Data Access Request protocol. With regard to the current CIFASD4 projects and the Central Repository, the IUPUI team is actively looking over and assessing the completeness and compatibility of received data dictionaries and test data files for each project. The AdminC also discussed opportunities for administrative supplements with select CIFASD PIs for obtaining additional resources that could expedite the release of tools and information into clinical practice. The AdminC assisted by scheduling and participating in these special teleconference discussions in October 2018 and assisted with the review of these applications.

Final preparations are currently underway for the next CIFASD4 face-to-face meeting being held in Vancouver, Canada in conjunction with the 8th International Conference on FASD on March 8, 2019. CIFASD will be providing a half-day plenary session at the 3-day conference. Organized by Drs. Charness and Riley, who also serve on the conference's Expert Planning Committee, the CIFASD-themed plenary is broken down into three segments with multiple CIFASD investigators presenting in each: Genetics (Eberhart, Fernandes, Parnell and Foroud), Biomarkers and Risk (Miranda, Weinberg and Chambers) and Automating Diagnosis and Treatment (Mattson, Suttie, Petrenko and Tapparello). An introduction and overview of CIFASD will be provided by Dr. Riley; he will also participate in the Diagnostics and Intervention sessions on the pre-conference day "Let's Talk" sessions. Additional consortium investigators are chairing and presenting in other plenaries (e.g., Coles and Foroud on their adult and genetic registries). As the majority of consortium PIs will be providing updates on their research throughout their plenary presentations, the agenda for the closed CIFASD meeting will include presentations by associated investigators Todd Coleman on wireless wearable sensors and Miguel del Campo on telemedicine pilot data. The rest of the agenda is still being determined.

Formal project evaluations by the Advisory Committee will be collected following the meeting. Members will be provided with the written progress reports, the slides from the recent mid-year project update WebEx presentations and snapshots of the current progress tracking tables. Evaluations will be collated and disseminated to each PI.

The AdminC is responsible for establishing, executing and maintaining a tracking system designed to dynamically monitor all CIFASD4 project progress and deliverables in a clear and concise manner. The AdminC provided final refinement instructions at the June 2018 face-to-face meeting and worked individually with all PIs to finalize each data table; it is responsible for any ongoing adjustments as projects move into further phases of their studies. The system went live in October 2018 and project PIs are responsible for ensuring their data tables are updated prior to each monthly meeting to accurately depict their project's data collection and task completion percentages. Ongoing reminders are sent out by the AdminC and assistance is provided as needed. All CIFASD investigators and Advisory Committee members have anytime access to view the secured spreadsheet housed within Google Drive. The AdminC also ensures the that CIFASD Publications Policy Protocol is being followed and provides monthly updates on its progress.

CIFASD continues to make an impact and maintain a visible presence at national and international scientific meetings. Six CIFASD investigators, including Drs. Riley and Charness, participated in a symposium entitled, "Predicting Outcomes of Fetal Alcohol Exposure in the CIFASD Cohort," at the RSA meeting held in San Diego, CA in June 2018. Additional CIFASD investigators were speakers in a symposium chaired by NIAAA advisor, Bill Dunty on early identification and screening of FASD. Students mentored by CIFASD investigators were up for and received awards (Enoch Gordis Research Recognition Award and Timothy A. Cudd Award) at the June 2018 RSA and FASDSG meetings. Dr. Riley also provided the introduction for Sarah Mattson as she received the FASDSG Henry Rosett Award. Several poster presentations on CIFASD findings were also on display in San Diego. Dr. Riley met with several research groups in town for RSA (e.g., Mack Mitchell and Mauro Ceccanti). At each meeting, he was able to discuss opportunities for potential collaborations with CIFASD investigators, the possibilities for integration with ongoing research and explore new joint endeavors. He also met with Dr. Charness face-to-face in San Diego in April and in June at RSA during a smaller meeting with other CIFASD investigators.

The 19th Congress of the International Society for Biomedical Research on Alcoholism (ISBRA) convened in Kyoto, Japan from September 9-13, 2018. The Congress attracted 500 registrants from 43 countries and despite Typhoon Jebi and the closure of a major airport in the area, the conference was well-attended. A CIFASD-focused symposium entitled, "CIFASD Studies on the Genetics of FASD," was included on the program with presentations by Drs. Parnell, Eberhart and Foroud. Dr. Riley served as the chair and discussant. RSA recently accepted a CIFASD-focused symposium for the June 2019 meeting to be held in Minneapolis, MN organized by Dr. Charness, also serving as the discussant. It is entitled, "CIFASD eMedicine to Scale the Diagnosis and Treatment FASD," and includes presentations by Drs. Riley, Suttie, Mattson and Petrenko. Other CIFASD investigators will present in a cross-center symposium on neuroimmune dysfunction and health outcomes of prenatal alcohol exposure organized by Joanne Weinberg.

Dr. Chockalingam, PI of the app development subaward, has continued his work with Dr. Mattson on her decision tree screening mobile app for use on phones and tablets and the web portal, including the incorporation of a field for the GUID. He also worked with Dr. Riley on the development of an automated Pullman spiral application. He is currently exploring the ability to automate other tools used in the diagnosis of FASD into mobile platforms. Dr. Riley routinely meets with Dr. Chockalingam to discuss progress.

A carryover request was submitted in mid-November 2018 and approved with a revised notice of grant award in mid-December. Funds were approved to increase the amount of effort for Dr. Riley and to extend additional funds to the Blue Resonance, LLC app development subaward. These funds improve upon and increase the possibility for CIFASD to incorporate and generate new eHealth technologies to broaden the reach of CIFASD studies and to research further expansion opportunities. The funds also allow for Dr. Chockalingam to travel to Vancouver for the March 2019 face-to-face meeting of CIFASD4.

With regard to the allocation of shared consortium resources, the AdminC shipped a portable Canfield Vectra 3D handheld Canon camera and laptop to Christie Petrenko in Rochester, NI in October 2018. The other handheld 3D camera and laptop still reside with Jeff Wozniak at UMN and the Bellus3D face camera tool and Huawei tablet are with Mike Suttie at the University of Oxford.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	Suttie M, Wozniak JR, Parnell SE, Wetherill L, Mattson SN, Sowell ER, Kan E, Riley EP, Jones KL, Coles C, Foroud T, Hammond P. Combined Face-Brain Morphology and Associated Neurocognitive Correlates in Fetal Alcohol Spectrum Disorders. Alcoholism, clinical and experimental research. 2018 September;42(9):1769-1782. PubMed PMID: 29935097; PubMed Central PMCID: PMC6120799.
Complete	Bodnar TS, Raineki C, Wertelecki W, Yevtushok L, Plotka L, Zymak-Zakutnya N, Honerkamp-Smith G, Wells A, Rolland M, Woodward TS, Coles CD, Kable JA, Chambers CD, Weinberg J. Altered maternal immune networks are associated with adverse child neurodevelopment: Impact of alcohol consumption during pregnancy. Brain, behavior, and immunity. 2018 October;73:205-215. PubMed PMID: 29738852; PubMed Central PMCID: PMC6344127.
Complete	Doyle LR, Moore EM, Coles CD, Kable JA, Sowell ER, Wozniak JR, Jones KL, Riley EP, Mattson SN. Executive Functioning Correlates With Communication Ability in Youth With Histories of Heavy Prenatal Alcohol Exposure. Journal of the International Neuropsychological Society : JINS. 2018 November;24(10):1026-1037. PubMed PMID: 30322415; PubMed Central PMCID: PMC6237635.
In Process at NIHMS	Barrett CE, Kable JA, Madsen TE, Hsu CC, Coles CD. The Use of Functional Near-Infrared Spectroscopy to Differentiate Alcohol-Related Neurodevelopmental Impairment. Developmental neuropsychology. 2019 January 20;:1-17. PubMed PMID: 30661412.
In Process at NIHMS	Doyle LR, Coles CD, Kable JA, May PA, Sowell ER, Jones KL, Riley EP, Mattson SN. Relation between adaptive function and IQ among youth with histories of heavy prenatal alcohol exposure. Birth defects research. 2019 February 4. PubMed PMID: 30719847.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Category	Explanation
Audio or video, Research Material, Educational aids or curricula, Data or Databases	http://cifasd.org – This is the website for CIFASD which contains various sections accessible by the public and an area reserved for individuals affiliated with CIFASD. The public sections contain descriptions of the projects, recent news and events in the field of FASD, a list of resources, an educational section containing videos made in conjunction with NOFAS and a slide set on FASD for educational use created from contributions from consortium investigators, a listing of CIFASD publications, and information and forms regarding data sharing. Within the secure area of the site, consortium members can access forms and protocols/concept sheets, the Central Repository, the publication policy and forms, progress reports, meeting recordings and other information directly relevant to CIFASD investigators. CIFASD data sharing policies, forms and data dictionary definitions are available for the public to view in the Data Sharing section of the CIFASD website. External investigators can utilize these materials to request permission to access research data from previous phases of CIFASD. Requests are reviewed and processed by the Data Access Committee of CIFASD.

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Specific Aim #1: Assure consistency as well as accuracy in recognition of fetal alcohol spectrum disorders (FASD) at all CIFASD project sites where new subjects are being recruited.
To better understand the full range of FASD features the Dysmorphology Research Resource will use the established CIFASD physical examination protocol and classification system to perform and/or validate physical examinations of all infants, children, and adults who are participants in the CIFASD renewal project who have not previously been examined by the Dysmorphology Research Resource examination team. Additionally, our team will use the established CIFASD examination training protocol to provide on-going training and re-training of local pediatricians, neonatologists, and geneticists who are providing preliminary examinations at select CIFASD sites. We will collaborate with the following U01 research projects:
•Early Predictors of FASD in Ukraine, PI Dr. Christina Chambers
•Multi-Modal Connectivity Methods for the Validation of Fetal Alcohol Spectrum Disorder Diagnostic Criteria, PI Dr. Jeff Wozniak
•A Multisite Neurobehavioral Assessment of Fetal Alcohol Spectrum Disorders, PI Dr. Sarah Mattson
•Image Analysis of Neurofacial Effects of Prenatal Alcohol Exposure, PI Dr. Peter Hammond
•Fetal Alcohol Spectrum Disorders in Adults: Health and Neurobehavior, PI Dr. Claire Coles
•Development and Evaluation of an Evidence-Based Mobile Health Caregiver Intervention for FASD, PI Dr. Christie Petrenko
•Immune dysregulation in FASD: Programming of Health and Neurobehavioral Outcomes, PI Dr. Joanne Weinberg

Specific Aim #2: To further develop and refine the telemedicine approach developed in the last funding period, we will expand upon our telemedicine capabilities in order to 1) reach children in underserved areas that lack access to physicians with expertise in clinical recognition of FASD, 2) train additional physicians in the clinical identification of FASD and 3) recruit additional research subjects who have been prenatally exposed to alcohol.
For this specific aim the Dysmorphology Research Resource will:
•Continue to work in collaboration with Dr. Jeff Wozniak at the University of Minnesota as well as with the Minnesota Organization on Fetal Alcohol Syndrome: MOFAS to improve upon the telemedicine program developed and field tested by the Dysmorphology Research Resource in the last funding period.
•Expand the telemedicine program in Minnesota and establish a new telemedicine program in New Mexico to diagnose FAS at Indian Health Hospitals and Clinics in remote areas throughout the state.
•Identify patients prenatally exposed to alcohol that may be eligible for participation in the CIFASD registry and refer to the registry, once functional.

Specific Aim #3: Contribute to the CIFASD Consortium research studies by utilizing and expanding upon the San Diego FASD research subject pool established at Rady Children's Hospital-San Diego
For this specific aim the Dysmorphology Research Resource will:
•Identify children prenatally exposed to alcohol to participate in the established San Diego FASD Research Subject Pool and obtain permission from participants to be contacted to participate in future CIFASD research studies.

B.1.a Have the major goals changed since the initial competing award or previous report?

Yes, Specific Aim 2

Revised goals:

Yes. A number of questions were raised about the Telemedicine Project within the DRR. It was suggested that measures of reliability and convergent validity are important to the evaluation of telemedicine as a viable option for diagnosis. It was also suggested that more clarity on the role of telemedicine as a training tool was needed, and further detail about the applicability of telemedicine as an approach in remote or underserved areas. In response to these overall concerns, we provide a more detailed description of the telemedicine approach and evaluation measures below. We adjusted the major goals outlined in Specific Aim 2 to address these issues. These changes were approved before we received the NOA but were not included in the last progress report.

Specific Aim #1: Assure consistency as well as accuracy in recognition of fetal alcohol spectrum disorders (FASD) at all CIFASD project sites where new subjects are being recruited.
To better understand the full range of FASD features the Dysmorphology Research Resource will use the established CIFASD physical examination protocol and classification system to perform and/or validate physical examinations of all infants, children, and adults who are participants in the CIFASD renewal project who have not previously been examined by the Dysmorphology Research Resource examination team. Additionally, our team will use the established CIFASD examination training protocol to provide on-going training and re-training of local pediatricians, neonatologists, and geneticists who are providing preliminary examinations at select CIFASD sites. We will collaborate with the following U01 research projects:
•Early Predictors of FASD in Ukraine, PI Dr. Christina Chambers
•Multi-Modal Connectivity Methods for the Validation of Fetal Alcohol Spectrum Disorder Diagnostic Criteria, PI Dr. Jeff Wozniak
•A Multisite Neurobehavioral Assessment of Fetal Alcohol Spectrum Disorders, PI Dr. Sarah Mattson
•Image Analysis of Neurofacial Effects of Prenatal Alcohol Exposure, PI Dr. Peter Hammond
•Fetal Alcohol Spectrum Disorders in Adults: Health and Neurobehavior, PI Dr. Claire Coles
•Development and Evaluation of an Evidence-Based Mobile Health Caregiver Intervention for FASD, PI Dr. Christie Petrenko
•Immune dysregulation in FASD: Programming of Health and Neurobehavioral Outcomes, PI Dr. Joanne Weinberg
Specific Aim #2: As the telemedicine approach to the diagnosis of FASD has not been previously demonstrated to be either a valid or

reliable measure, these hypotheses must first be tested. In addition, telemedicine as a training tool must be tested prior to implementing this mode of evaluation in remote or underserved areas. To accomplish this, we propose to do the following:
•Convergent Validity of Telemedicine: This construct represents the degree to which there is a positive correlation between the diagnostic classification derived from a face-to-face physical examination conducted by an expert dysmorphologist, and a telemedicine evaluation conducted by an expert dysmorphologist for the same child. To test this hypothesis, we will perform both a face-to-face exam and a telemedicine exam for a sample of children from one CIFASD site in San Diego (San Diego research subject pool). In Years 1 through 2, a minimum of 16 children with FAS and 32 children without FAS seen by one of the two DRR dysmorphologists, Dr. Ken Jones or Dr. Miguel del Campo, at one of the two sites will also be evaluated by the other dysmorphologist using the telemedicine approach. Although ideally, the same dysmorphologist would perform the exam for a given child using both modes of evaluation, this would introduce potential bias due to prior knowledge of the result of the face-to-face exam before performing the telemedicine exam. Instead, the two dysmorphologists will perform equal numbers of examinations using the telemedicine and face-to-face mode so that any dysmorphologist-specific differences would at least be balanced across the two types of assessments. Convergent validity between the two modes of evaluation will be tested using the Kappa statistic for concordance. With respect to power, if we have at least good agreement (70% or higher), we will have a sufficient sample size with 48 children. Any non-concordant evaluations will be further assessed for possible reasons for lack of congruence, e.g., the age of the child, quality of images, etc.
•Reliability of Telemedicine: This construct represents the degree to which there is consistency in the result of the telemedicine exam mode when repeatedly applied using the same of different examiners. In other words, is the diagnostic classification highly replicable using this technology. To test this hypothesis, in Years 2 through 4, we will build this into the planned training component of the telemedicine project (see the following bullet point). At the San Diego site, pediatric residents who are selected for training on the diagnosis, after basic hands-on training by Dr. Ken Jones or Dr. Miguel del Campo, will perform face-to-face examinations of 16 FAS and 16 non-FAS children, while at the same time having the child evaluated remotely and independently by Dr. Jones and then by Dr. del Campo using telemedicine equipment. The reliability of telemedicine classifications assigned by Dr. Jones compared to Dr. del Campo will be evaluated using the Kappa statistic for concordance. With respect to power, if we have a very good agreement (90%), we will have a sufficient sample size with 32 children. Any diagnoses that are discordant across the two dysmorphologist will be further evaluated regarding causes of the discrepancies, e.g., the experience of the trainee, age of the child etc.
•Training via Telemedicine: In Years 2 through 4, five pediatric residents per year at the San Diego site will be selected for training. After an initial orientation and viewing of the DRR training DVDs, the resident trainees will work with one of the DRR dysmorphologists to learn to perform examinations in person. Once the initial training phase is completed, the residents, as described above, will perform face-to-face examinations of new children while consulting with Drs. Jones and del Campo by telemedicine. At the completion of training of each resident, they will be asked to complete a survey regarding the training process, the value of the DVD, the utility of the telemedicine consultation approach, and level of confidence in making a diagnosis of FAS or not.
•Application of Telemedicine in Remote Areas: In Year 5, after completion of above-listed items, the telemedicine approach will be tested in at least one remote clinic in Minnesota and one in New Mexico. Using the protocol developed for the training mentioned above, we will train one or more physicians in these remote clinics to perform the examination. We will then move to telemedicine as an approach in both sites to providing expert consultation from San Diego for clinicians at the remote sites. As it is unknown what the actual mix of patients will be at these sites with respect to numbers with FAS vs not, the process will be evaluated at the end of Year 5 and a determination made as to whether this is a viable, cost-effective, and value-added option at these sites.
Specific Aim #3: Contribute to the CIFASD Consortium research studies by utilizing and expanding upon the San Diego FASD research subject pool established at Rady Children's Hospital-San Diego
For this specific aim the Dysmorphology Research Resource will:
•Identify children prenatally exposed to alcohol to participate in the established San Diego FASD Research Subject Pool and obtain permission from participants to be contacted to participate in future CIFASD research studies.

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

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B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

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B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

Interim results have been presented via Skype and in person to the research teams in Ukraine and at training meetings for general groups of psychologists, educators, and physicians in Ukraine.

Poster Abstracts and Presentations
Abstracts
1. Doyle LR, Chambers CD, Jones KL, Mattson SN, the CIFASD. Validation of a decision tree for clinical identification of children affected by prenatal alcohol exposure in a low-risk sample. Alcohol Clin Exp Res. 2018;42(S1):115A. Presented at the 41st Annual Scientific Meeting of the Research Society on Alcoholism. San Diego, CA, June 2018. (Poster)

Presentations
1. Keynote address at David W. Smith Workshop, A retrospective look at 45 years of the fetal alcohol spectrum disorder: How did we get here from where we started, 8/25/19.
2. FASD talk in Ukraine, 9/17/18
3. March of Dimes Annual Meeting a talk on Prevention of FASD in Irvine, CA, 11/5/18
4. FASD talk to Los Angeles federal public defenders, 11/7/18
5. FASD talk to undergraduate students at UCSD, 11/29/18

Symposia
1. Suttie M, Wetherill L, Jacobson SW, Hacobson JL, Hoyme EH, Mattson S, Sowell ER, Coles C, Wozniak JR, Riley EP, Jones KL, Mukherjee R, Foroud T, Hammond P. Using 3D facial analysis to identify minor facial anomalies and ethnic differences in effects of prenatal alcohol exposure. Alcohol Clin Exp Res. 2018;42(S1):247A. Presented at the 41st Annual Scientific Meeting of the Research Society on Alcoholism. San Diego, CA, June 2018. (Symposium)

2. Coles CD, Kable JA, Mesa DA, Coleman TP, Jones KL, Yevtushok L, Kulikovsky Y, Wertelecki W, Chambers CD. Early identification of effects of prenatal alcohol exposure: infant cardiac orienting response as a biomarker. Alcohol Clin Exp Res. 2018;42(S1). Presented at the 41st Annual Scientific Meeting of the Research Society on Alcoholism. San Diego, CA, June 2018. (Symposium)

3. Suttie M, Wetherill L, Jacobson SW, Hacobson JL, Hoyme EH, Mattson S, Sowell ER, Coles C, Wozniak JR, Riley EP, Jones KL, Mukherjee R, Foroud T, Hammond P. Using 3D facial analysis to identify minor facial anomalies and ethnic differences in effects of prenatal alcohol exposure. Alcohol Clin Exp Res. 2018;42(S1):247A. Presented at the 41st Annual Scientific Meeting of the Research Society on Alcoholism. San Diego, CA, June 2018. (Symposium)

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

We plan to do the following:

Aim 1: We will continue to see new patients recruited from Dr. Mattson's U01 in San Diego and Dr. Wozniak's U01 in Minneapolis. We will see subjects recruited by Dr. Coles U01 in Atlanta and Seattle and Dr. Weinberg's U01 in Vancouver when we are requested to do so.

Aim 2: In Years 2 through 4 we will investigate the reliability of telemedicine. Also in Years 2 through 4 we will train pediatric residents at UCSD in the utility of the telemedicine consultation approach and their level of confidence in diagnosing FASD or not. In Year 5 we will test the telemedicine approach in underserved, remote, communities in the United States.

Aim 3: We will continue to recruit subjects for our CIFASD consortium research registry in order to provide the majority of subjects for the clinical project in CIFASD.

B.2 Accomplishments

Aim 1. Assure consistency as well as accuracy in recognition of fetal alcohol spectrum disorders (FASD) at all CIFASD project sites where new subjects are being recruited.

1) Major Activities. Between 06/01/2018 and 02/22/2019, using the CIFASD physical examination protocol and classification system, Dr. Jones performed 26 infant/children physical examinations in San Diego, CA and 35 in Minneapolis, MN. Drs. del Campo and Jones have trained 7 Pediatric Residents and 1 Fellow in the Genetics and Dysrmophology Clinic at Rady Children's Hospital- San Diego to perform physical examinations on children with and without fetal alcohol syndrome. These trainings occurred in four full day FAS clinics per month by Dr. Jones and two full day FAS clinics per month by Dr. del Campo.

2) Specific Objectives. Use the established CIFASD physical examination protocol and classification system to perform and/or validate physical examinations of all infants, children, and adults who are participants in the CIFASD renewal project who have not previously been examined by the Dysmorphology Research Resource examination team.

Use the established CIFASD examination training protocol to provide on-going training and re-training of local pediatricians, neonatologists, and geneticists who are providing preliminary examinations at select CIFASD sites.

3) Significant Results. 26 completed physical examinations at the San Diego, CA, site. Drs. del Campo and Jones have trained 7 Pediatric Residents and 1 Fellow in the Genetics and Dysrmophology Clinic at Rady Children's Hospital- San Diego to perform physical examinations on children with and without fetal alcohol syndrome.

4) Key Outcomes or Other Achievements. Completed dysmorphology exams and trainings.

Aim 2. Convergent Validity (to be completed by May 2019). 46 children including those from 2 months of age to 12 years of age have been evaluated by Drs. Jones and del Campo. 16 of these have been FAS or PFAS cases and 31 have been controls. By May 2019 we will complete all additional 5 controls. For each child four separate examinations were completed, 2 by each physician.

1) Major Activities. 46 children including those from 2 months of age to 12 years of age have been evaluated by Drs. Jones and del Campo. 16 of these have been FAS or PFAS cases and 31 have been controls.

2) Specific Objectives. Further develop and refine the telemedicine approach developed in CIFASD III to expand upon our telemedicine capabilities in order to reach children in underserved areas that lack access to physicians with expertise in clinical recognition of FASD.

3) Significant Results. 46 children including those from 2 months of age to 12 years of age have been evaluated by Drs. Jones and del Campo. 16 of these have been FAS or PFAS cases and 31 have been controls.

4) Key Outcomes or Other Achievements.

Aim 3. Contribute to the CIFASD Consortium research studies by utilizing and expanding upon the San Diego FASD research subject pool established at Rady Children's Hospital-San Diego.

1) Major Activities. Between 06/01/2018 and 02/22/2019, through the FASD Clinic at RCHSD, we have enrolled 48 new subjects (22 males, 1 transgender, 25 females) into the FASD Research Subject Pool bringing our total registry numbers up to 202. We have referred 48 subjects from this research pool to Dr. Sarah Mattson's Decision Tree project. Additionally, to support Dr. Joanne Weinberg's project, we have recently obtained IRB approval to contact subjects in the FASD Research Subject Pool in order to consent them and collect 2 ML of whole blood, from which 0.25 mL's of plasma will be shipped to Dr. Weinberg's lab using the appropriate materials transfer agreement. Three have been collected but none have been sent. We sent 1 plasma sample to Hashimoto Kazue-Torii (Children's National Medical Center).

2) Specific Objectives. Support the CIFASD Consortium research studies by utilizing and expanding upon the San Diego FASD research subject pool established at Rady Children's Hospital-San Diego.

3) Significant Results. We have enrolled 48 new subjects (22 males, 1 transgender, 25 females) into the FASD Research Subject Pool bringing our total registry numbers up to 202. In addition, we have referred 48 subjects from this research pool to Dr. Sarah Mattson's Decision Tree project. We have submitted an IRB application to support Dr. Joanne Weinberg's project and have received approval. Three have been collected but none have been sent. We sent 1 plasma sample to Hashimoto Kazue-Torii (Children's National Medical Center).

4) Key Outcomes or Other Achievements. FASD Research Subject Pool registry numbers are increasing and CIFASD research studies are being supported through our referrals.

The table below shows our Phase IV (CIFASD4) numbers as of February 22, 2019.

Category	**N**
Number of in person CIFASD completed dysmorphology examinations by location	Atlanta, GA = 0 Minneapolis, MN = 35 San Diego, CA = 26 Seattle, WA = 0 Ukraine = 0 Vancouver, BC = 0
Number of physicians trained using the CIFASD examination training protocol	Total =7
Number subjects recruited into the FASD research registry	Male = 22 Female =25 Transgender= 1 Total for the reporting period = 48 Total in registry = 202
Telemedicine	Minnesota & San Diego (convergent validity of telemedicine **Yrs. 1-2**): Children with FAS = 14 Controls = 35 The information below will be collected in Yrs. 2-5 and therefore, there is nothing to report at this time San Diego (reliability of telemedicine **Yrs. 2-4**): Children with FAS = Controls = San Diego (training telemedicine **Yrs. 2-4**): Pediatric residents trained = 0 Minnesota & New Mexico (application of telemedicine in remote areas **Yr. 5**): Telemedicine approach tested in remote site in MN = y/n Number of Physicians trained in remote site in MN = Telemedicine approach tested in remote site in NM = y/n Number of Physicians trained in remote site in NM =
Other CIFASD Shared Data	Weinberg U01 (**Yrs. 1-5**) Number of plasma samples sent = 0 (3 Collected but not sent) Mattson U01 (**Yrs. 1-5**) Number of referrals made = 48 The information below will be collected in Yrs. 3-5 and therefore, there is nothing to report at this time Petrenko U01 (**Yrs. 3-5**) Number of referrals made = Hammond U01 (**Yrs. 3-5**) Number of physical examinations performed using telemedicine to validate automated face screening tool =

B.4 TRAINING AND PROFESSIONAL DEVELOPMENT
During this reporting period, Dr. Jones and Dr. del Campo have trained Pediatricians, Geneticists and Neonatologists who attend his FAS clinic at Rady Children's Hospital-San Diego.

The training video created in CIFASD III titled "Diagnosis of the Fetal Alcohol Syndrome," (https://www.youtube.com/watch?v=yP9_qzGqzqk&feature=youtu.be) is still available on YouTube and has been viewed by 97 people.

Describe your project's interrelation with aims of the CIFASD consortium its other projects and the progress on the interactions.

The overarching goal of the Dysmorphology Research Resource is to use the previously established CIFASD physical examination protocol and classification system to perform and/or validate physical examinations of all infants, children and adults who are participants in the CIFASD renewal projects and who have not previously been examined by the Dysmorphology Research Resource examination team. Through this process, the Dysmorphology Research Resource team will collaborate with other consortium projects to better understand the full range of features associated with FASD.

For CIFASD4, the Dysmorphology Research Resource will collaborate with the following U01 investigators in that all children will be evaluated using a standard protocol by one of two physicians (Dr. Jones and Dr. del Campo) with expertise in recognition of features characteristic of prenatal alcohol exposure dramatically increases the integrity of conclusions that can be drawn regarding the overall aims of the CIFASD Consortium:

- Dr. Christina Chambers (Early Predictors of FASD in Ukraine)
- Dr. Jeff Wozniak (Multi-Modal Connectivity Methods for the Validation of Fetal Alcohol Spectrum Disorder Diagnostic Criteria) in Minnesota
- Dr. Sarah Mattson (A Multisite Neurobehavioral Assessment of Fetal Alcohol Spectrum Disorders) in San Diego, CA and Minneapolis, MN
- Dr. Claire Coles (Fetal Alcohol Spectrum Disorders in Adults: Health and Neurobehavior) in Atlanta, GA and Seattle, WA
- Dr. Christie Petrenko (Development and Evaluation of an Evidence-Based Mobile Health Caregiver Intervention for FASD) in Rochester, NY, San Diego, CA, and Minneapolis, MN
- Dr. Joanne Weinberg (Immune dysregulation in FASD: Programming of Health and Neurobehavioral Outcomes) in San Diego, CA
- Dr. Peter Hammond (Image Analysis of Neurofacial Effects of Prenatal Alcohol Exposure) in Oxford, England

In addition, the Dysmorphology Research Resource through the FASD Research Subject Pool will inform parents of qualified subjects of Dr. Petrenko's study and if they agree, will refer them to Dr. Petrenko. These referrals will not take place until 2020 (Year 3 of the study).

The Dysmorphology Research Resource has been re-contacting the parents of eligible children who are enrolled in the FASD Research Subject Pool to ask permission for their child to participate in Dr. Weinberg's study by giving 2ML of whole blood, and completing an age appropriate questionnaire. A total of 0.25mL aliquot of plasma from each subject is shipped under the appropriate materials transfer agreement to Dr. Weinberg's lab at the University of British Columbia.

Inclusion Enrollment Report

Inclusion Data Record (IDR) #: 174064

Using an Existing Dataset or Resource: No

Delayed Onset Study ?: No

Clinical Trial: No

Enrollment Location: Foreign

NIH Defined Phase III Clinical Trial: No

Study Title: Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD) Dysmorphology Research Resource (U24) - Foreign

Planned Enrollment

Comments: The Dysmorphology Research Resource will collaborate with two sites in Ukraine,one site in Canada, five sites involved with U01 projects in the U.S. as well as remote outlying clinics in Minnesota and New Mexico This Enrollment Report includes the two sites in Ukraine and the one site in Canada.

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/Alaska Native	0	0		0	0					**0**
Asian	0	0		0	0					**0**
Native Hawaiian or Other Pacific Islander	0	0		0	0					**0**
Black or African American	1	2		0	0					**3**
White	112	113		6	6					**237**
More than One Race	0	0		0	0					**0**
Unknown or Not Reported										
Total	**113**	**115**		**6**	**6**					**240**

Cumulative Enrollment

Comments: This Enrollment Report includes the two sites in Ukraine and the one site in Canada.

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/Alaska Native	0	0	0	0	0	0	0	0	0	**0**
Asian	0	0	0	0	0	0	0	0	0	**0**
Native Hawaiian or Other Pacific Islander	0	0	0	0	0	0	0	0	0	**0**
Black or African American	0	0	0	0	0	0	0	0	0	**0**
White	0	0	0	0	0	0	0	0	0	**0**
More than One Race	0	0	0	0	0	0	0	0	0	**0**
Unknown or Not Reported	0	0	0	0	0	0	0	0	0	**0**
Total	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Inclusion Data Record (IDR) #: 174065

Using an Existing Dataset or Resource: No

Delayed Onset Study ?: No

Clinical Trial: No

Enrollment Location: Domestic

NIH Defined Phase III Clinical Trial: No

Study Title: Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD) Dysmorphology Research Resource (U24) - Domestic

Planned Enrollment

Comments: This Enrollment Report includes the five sites in the United Stated, including Minneapolis, MN, San Diego, CA, Atlanta, GA, Seattle, WA, and Rochester, NY, plus the remote, Public Health and Indian Health clinics in Minnesota and New Mexico.

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/Alaska Native	133	133		0	0					**266**
Asian	12	12		0	0					**24**
Native Hawaiian or Other Pacific Islander	0	0		0	0					**0**
Black or African American	84	84		28	28					**224**
White	175	174		24	25					**398**
More than One Race	0	0		0	0					**0**
Unknown or Not Reported										
Total	**404**	**403**		**52**	**53**					**912**

Cumulative Enrollment

Comments: This Enrollment Report includes the five sites in the United Stated, including Minneapolis, MN, San Diego, CA, Atlanta, GA, Seattle, WA, and Rochester, NY, plus the remote, Public Health and Indian Health clinics in Minnesota and New Mexico.

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/Alaska Native	0	0	0	0	0	0	0	0	0	**0**
Asian	3	0	0	0	0	0	0	0	0	**3**
Native Hawaiian or Other Pacific Islander	0	0	0	0	0	0	0	0	0	**0**
Black or African American	4	1	0	0	0	0	0	0	0	**5**
White	4	3	0	2	3	0	0	0	0	**12**
More than One Race	0	1	0	0	0	0	0	0	0	**1**
Unknown or Not Reported	0	0	0	0	0	0	0	0	0	**0**
Total	**11**	**5**	**0**	**2**	**3**	**0**	**0**	**0**	**0**	**21**

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	Suttie M, Wozniak JR, Parnell SE, Wetherill L, Mattson SN, Sowell ER, Kan E, Riley EP, Jones KL, Coles C, Foroud T, Hammond P. Combined Face-Brain Morphology and Associated Neurocognitive Correlates in Fetal Alcohol Spectrum Disorders. Alcoholism, clinical and experimental research. 2018 September;42(9):1769-1782. PubMed PMID: 29935097; PubMed Central PMCID: PMC6120799.
Complete	Doyle LR, Moore EM, Coles CD, Kable JA, Sowell ER, Wozniak JR, Jones KL, Riley EP, Mattson SN. Executive Functioning Correlates With Communication Ability in Youth With Histories of Heavy Prenatal Alcohol Exposure. Journal of the International Neuropsychological Society : JINS. 2018 November;24(10):1026-1037. PubMed PMID: 30322415; PubMed Central PMCID: PMC6237635.
In Process at NIHMS	Doyle LR, Coles CD, Kable JA, May PA, Sowell ER, Jones KL, Riley EP, Mattson SN. Relation between adaptive function and IQ among youth with histories of heavy prenatal alcohol exposure. Birth defects research. 2019 February 4. PubMed PMID: 30719847.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Nothing to report

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? No

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization?

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

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

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

***Poster Abstracts and Presentations**

Abstracts

1. Doyle LR, Chambers CD, Jones KL, Mattson SN, the CIFASD. Validation of a decision tree for clinical identification of children affected by prenatal alcohol exposure in a low-risk sample. Alcohol Clin Exp Res. 2018;42(S1):115A. Presented at the 41st Annual Scientific Meeting of the Research Society on Alcoholism. San Diego, CA, June 2018. (Poster)

Presentations

1. Keynote address at David W. Smith Workshop, A retrospective look at 45 years of the fetal alcohol spectrum disorder: How did we get here from where we started, 8/25/19.
2. FASD talk, Ukraine, 9/17/18
3. March of Dimes Annual Meeting a talk on Prevention of FASD, Irvine, CA, 11/5/18
4. FASD talk to Los Angeles federal public defenders, 11/7/18
5. FASD talk to undergraduate students at UCSD, 11/29/18

Symposia

1. Suttie M, Wetherill L, Jacobson SW, Hacobson JL, Hoyme EH, Mattson S, Sowell ER, Coles C, Wozniak JR, Riley EP, Jones KL, Mukherjee R, Foroud T, Hammond P. Using 3D facial analysis to identify minor facial anomalies and ethnic differences in effects of prenatal alcohol exposure. Alcohol Clin Exp Res. 2018;42(S1):247A. Presented at the 41st Annual Scientific Meeting of the Research Society on Alcoholism. San Diego, CA, June 2018. (Symposium)

2. Coles CD, Kable JA, Mesa DA, Coleman TP, Jones KL, Yevtushok L, Kulikovsky Y, Wertelecki W, Chambers CD. Early identification of effects of prenatal alcohol exposure: infant cardiac orienting response as a biomarker. Alcohol Clin Exp Res. 2018;42(S1). Presented at the 41st Annual Scientific Meeting of the Research Society on Alcoholism. San Diego, CA, June 2018. (Symposium)

3. Suttie M, Wetherill L, Jacobson SW, Hacobson JL, Hoyme EH, Mattson S, Sowell ER, Coles C, Wozniak JR, Riley EP, Jones KL, Mukherjee R, Foroud T, Hammond P. Using 3D facial analysis to identify minor facial anomalies and ethnic differences in effects of prenatal alcohol exposure. Alcohol Clin Exp Res. 2018;42(S1):247A. Presented at the 41st Annual Scientific Meeting of the Research Society on Alcoholism. San Diego, CA, June 2018. (Symposium)

Jones U24 Dysmorphology Core	Current Month	Cumulative Total	May 2019 Goal	% to Goal	Overall Goal	Completion Goal Date	May 2018 Goal	May 2019 Goal	May 2020 Goal	May 2021 Goal	May 2019 Goal
In Person Dysmorphology Exams Completed											
Atlanta	0	0	24	0.0%		5/31/2022	0	24	60	94	
Minnesota	0	40	60	66.7%		5/31/2020	30	60	90	90	
Seattle	0	0	24	0.0%		5/31/2022	0	24	60	94	
San Diego	0	74	100	74.0%		5/31/2021	40	100	160	220	
Ukraine	0	5		#DIV/0!		5/31/2022					
Vancouver	0	3		#DIV/0!		5/31/2021					
Telemedicine											
Minnesota (convergent validity)	0	0	48	0.0%	49	5/31/2020					49
San Diego (convergent validity)	0	46	48	95.8%	49	5/31/2020					49
San Diego (reliability of telemedicine)	0	0	32	0.0%	33	5/31/2021					33
San Diego (training telemedicine)	0	0	15	0.0%	16	5/31/2021					16
Minnesota (application in remote areas)	0	0	0	#DIV/0!	1	5/31/2022					1
New Mexico (application in remote areas)	0	0	0	#DIV/0!	1	5/31/2022					1
Oxford, England	0	0	0	#DIV/0!	1	5/31/2022					1
Rochester	0	0	0	#DIV/0!	1	5/31/2022					1
San Diego FASD Research Subject Pool											
Number of subjects recruited	48	202	400	50.5%	401	5/31/2022					401
Training											
Number of physicians trained	0	16	0	#DIV/0!	1	5/31/2022					1

	Start	End
Current month (defined by project) =	2/1/2019	2/28/2019
Date of project numbers update entry =	3/1/2019	

Cumulative - at the end of each year.

Jones U24 Dysmorphology Core

In Person Dysmorphology Exams Completed

- Minnesota = 40
- San Diego = 74
- Ukraine = 5
- Vancouver = 3

San Diego FASD Redsearch Subject Pool

- Mattson U01
- Hashimoto-Torii U01
- Weinberg U01

Number of Physicians Trained = 14

Jones U24 Dysmorphology Core (continued)

- Telemedicine

  Convergent Validity

  San Diego subject number = 23

- We have tested each subject using both the TES and a smart phone.
- For each subject both Jones and del Campo have done a face-to-face exam and a telemedicine exam.
- The smart phone takes less time, is easier to use, and is much less expensive that the TES.

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The current study, CIFASD Phase IV, builds on the existing prospective Ukraine cohort. We have added a new subset of the longitudinal cohort consisting of those children who completed participation in the preschool neurobehavioral evaluation in Phase III. We will also prospectively recruit an additional pregnancy/infant sample specifically to address Aims 1 and to address a portion of the work outlined for Aim 2.

Aim 1. Develop a panel of prenatal/infancy biomarkers that can predict FASD
Aim 1.a. Determine the positive and negative predictive value of selected maternal and infant miRNAs measured prior to birth and in early infancy as predictors of FASD outcome
Aim 1.b. Determine the positive and negative predictive value of the cardiac orienting response (COR) paradigm administered in early infancy as a predictor of FASD outcome
Aim 1.c. In collaboration with U01 PI J. Weinberg, determine the positive and negative predictive value of selected maternal and infant cytokines measured prior to birth and in early infancy as predictors of FASD outcome

Aim 2. Develop risk/resilience profiles based on early markers identified in Aim 1 and other factors that will adequately predict preschool and school age performance
Aim 2.a. Perform analysis of existing and newly obtained prospective cohort data to develop a prediction model for FASD outcomes in children using social, environmental, economic, health, and other available data
Aim 2.b. Expand the risk/resilience profiles developed in Aim 2.a. with biomarkers identified in Aim 1
Aim 2.c. Test the prediction model with the subset of factors identified in Aim 2.a. that are available from the CIFASD Phase II and III retrospective sample of children with and without FASD

Aim 3. Collaborate with others in the CIFASD consortium
Provide data and biological samples from the Ukraine cohort, along with analytical support to U01 PI Weinberg to assess markers of inflammation in mothers and their children who are or are not affected by FASD; provide data from the Ukraine cohort to U01 PI Mattson to test/adapt the FASD Decision Tree; provide data and biological samples from the Ukraine cohort and assist in the analyses for UH2 PI Torri to test the value of single cell technology in identifying specific epigenetic markers in infants/children with neurobehavioral impairment associated with prenatal alcohol exposure; provide 2D ultrasound fetal facial profiles and associated clinical data from the Ukraine cohort to U01 PI Hammond to test the hypothesis that these measures can assist with early identification of FASD-affected infants. Additional potential collaborations drawing on banked samples from the Ukraine cohort will be possible with U01 PI Foroud who is assessing the genetics of FASD, and UH2 PI Blanchard whose animal model will be examining the role of the gut microbiome in FASD

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

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B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

Yes

Revision/ Supplements #	Revision/ Supplements Title	Specific Aims	Accomplishments
3U01AA014835-15S1	Early Predictors of FASD in Ukraine	The purpose of this award is to support efforts to build on the existing aims of this longitudinal cohort study in Ukraine, which are focused on earlier identification of children affected by prenatal alcohol exposure, by addressing the potentially important confounder of comorbidity with HIV infection in pregnant women.	Pregnancy screening tool in use was modified to incorporate a specific question about HIV status and medication treatment. In discussions with the foreign site collaborators, it was determined that a more productive approach to examine the co-occurrence of alcohol and HIV is to assess women who receive services through a specialty clinic. So, in a 3rd site, we have identified where 800 children have been born to HIV positive mothers and data can be captured from a subset of 100 on co-occurrence of alcohol use in

			pregnancy. These data will be obtained in March-December 2019. Maternal blood samples have been collected on 57 of the 200 target sample size of pregnant enrollees at the 2 sites.

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

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B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

Findings have been presented via Skype and in person to the research teams in Ukraine and at training meetings for general groups of psychologists, educators and physicians in Ukraine held in September, 2018. Slides of publication findings have been provided to NOFAS. Publications have been highlighted in the NIAAA newsletter. Results have been presented at RSA 2018 meeting, accepted for Vancouver meeting symposium, accepted for Teratology Society symposium 2019, and submitted to RSA 2019.

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Aim 1: We will finish recruitment of new mothers, analyze initial shipment of maternal and infant samples and collect first wave of COR data. Under the supplement, we will analyze first wave of biomarkers of exposure data.
Aim 2: We will expand the individual clinical prediction model to incorporate school-age testing results. We will incorporate COR, and maternal biomarkers data into the models.
Aim 3: We will continue to work with other investigators in the Consortium

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

Aim 1. Develop a panel of prenatal/infancy biomarkers that can predict FASD
For this Aim, a new cohort of 200 pregnant women is proposed for recruitment and follow-up of mothers and their infants, and a subset of school age children previously enrolled in the study will completed a new neurobehavioral assessment at 7-10 years of age.

Aim 1. Biomarkers in Mothers and Children

1) **Major Activities.**

Three biomarkers in newly recruited mothers and infants are being assessed as part of Aim 1. Two of these biomarkers require blood samples (miRNA and markers of inflammation). To date, 57 new maternal blood samples in the mid-trimester have been obtained, and 21 new maternal blood samples in the third trimester have been obtained. Of the 11 infant who have already been born, we are still awaiting the first-year visit to capture blood samples. However, among school age children in the cohort, 32 blood samples have been collected from 7-10 year-olds. Due to the cost of international shipping of these samples, it is planned to ship the existing lot of 89+ samples to the U.S. in March, 2019, at which point aliquots will be shipped to Drs. Weinberg and Miranda for assays. In addition, from the FASD Research Resource in San Diego, 3 samples from children diagnosed with FASD have been collected and banked for future shipment to Drs. Miranda and Weinberg when the accumulated number is sufficient to run as a group. An additional 37 families enrolled in the Research Resource are currently being contacted to invite to participate in the blood sampling.
With respect to the third biomarker, the cardiac orienting response paradigm (COR), this measure can only be first captured when the newly recruited infants reach at least 6 months of age. None of the 11 infants already delivered in the newly recruited cohort have yet reached that first visit timepoint.
Under funding from a separate R01, the platform for wireless transmission and scoring is currently under development for the COR. The platform is planned to be mobilized in the field in Ukraine in May, 2019. This, along with a functional near infrared spectroscopy (fNIRS) measure also being deployed in the Ukraine sample under separate R01 funding, will add to the components of potential value in accomplishing this Aim.

2) **Specific Objectives.**

The objective of this Aim is to determine if these biomarkers individually or in combination can function as a specific and acceptably sensitive early marker of FASD affected infants.

3) **Significant Results, Developments of Conclusions.**

While recruitment has continued as planned (30% of target sample has been

recruited), additional analyses of existing miRNA data has continued. In Dr. Miranda's lab, additional work in placental cell culture and animal models has led to findings that maternal circulating miRNAs which predict adverse infant outcomes due to prenatal alcohol exposure also interfere with trophoblast growth and invasion resulting in diminished placental and fetal growth. These data provide an explanation for FASD-associated growth deficiency in the fetus/infant (Tseng et al, 2019 in press).

Aim 2. Develop risk/resilience profiles based on early markers identified in Aim 1 and other factors that will adequately predict preschool and school age performance

1) **Major Activities.**

We have built a prediction model using novel fast covariance estimation (FACE) with repeated measures of growth on weight, length and head circumference, along with LASSO to screen for clinical variables to predict infant performance on the Bayley Scales of Infant Development (Xu et al, 2019, submitted). We have subsequently more carefully examined the mediating role of gestational age at delivery on neurobehavioral performance preceded by prenatal alcohol (Coles et al, 2018), and we have developed a novel approach for categorizing drinking patterns over the course of pregnancy that are predictive of subsequent growth and performance which may have important utility in defining individual clinical risk/resilience (Bandoli et al, 2019). We have reclassified the children in the cohort who have been retested at preschool age with respect to FAS, pFAS, ARND and FASD by applying the Hoyme et al (2016) revised criteria for FASD. With these findings, we have extended the risk/resilience model to incorporate additional clinical and growth measures obtained from children between 12 months and 4-5 years of age to predict preschool performance on the preschool neurobehavioral testing battery.

2) **Specific Objectives.**

The objective of this Aim is to develop risk/resilience profiles that can be applied in clinical settings to best determine if an individual child is at higher vs lower risk of future deficits in performance.

3) **Significant Results, Developments of Conclusions.**

Our initial risk/resilience model including 440 mother-infant pairs performed alcohol-exposed infants with Areas Under the Curve (AUC) for Bayley performance >1 standard deviation below the mean ranging from 0.79 to 0.85, and amount of variance explained ranging from 22-25%. In the expanded model, using preschool performance in 305 children as the outcome, among alcohol-exposed, we also found that the AUC for the full model was 0.81 and 30% of the variance accounted for. Addition of previous Bayley scores into the same models containing growth and clinical variables did not improve performance. Finally, we

found that catch-up growth by 1 year in infants who were born small on weight or head circumference was associated with significantly better performance on the Bayley at 1 year, suggesting that capture of this specific pattern of growth is essential to incorporate into risk/resilience individual prediction models.

Aim 3. Collaborate with others in the CIFASD consortium

1) Major Activities.

1. Weinberg U01 - we have collected blood samples (78 maternal and 32 child) that will be shipped from Ukraine in March, 2019, and aliquots sent to Dr. Weinberg for Markers of Inflammation. We have collected 3 blood samples and associated Health Questionnaire data from children with FASD for Dr. Weinberg from the FASD Research Repository in San Diego and are contacting an additional 37 families eligible for this sampling.
2. Torii UH2 – we have collected 3 blood samples from children with FASD from the FASD Research Repository in San Diego and shipped these, same day, to Dr. Torii.
3. Suttie U01 – 3D camera at one site will capture images of school age children being tested.
4. Noble U01 – 2D ultrasounds have been captured for at least one time point for 56 newly enrolled pregnant women in the cohort. Three sample recordings have been sent to Dr. Noble for review.
5. Mattson U01– data shared with Dr. Mattson who is using these data to test and modify Decision Tree.

2) Specific Objectives.

The specific objectives of this Aim are to provide samples, data analysis support, and data to others in the Consortium

3) Significant Results, Developments of Conclusions.

Dr. Suttie has analyzed 113 3D images from infants and children in Ukraine. Using Bayley scores and facial features as outcomes, the preliminary analysis suggests that young infants' facial "grimacing" and other non-standard presentations present barriers to using 3D images to predict neurobehavioral performance or diagnostic category. Additional work for this same dataset will focus on the utility of the 3D images to classify children who exhibit deficits on the preschool testing battery.

Table of enrollment and samples for CIFASD4

Category	N
New Data (Aim 1)	
Mothers	
Enrolled	Moderately/heavily exposed= 30 (goal 120 Yrs. 1-3)

Category	N
	Low/unexposed= 29 (goal 80 Yrs. 1-3)
2D ultrasound on new mothers	Moderately/heavily exposed =28 Low/unexposed= 28
5 mL blood samples collected	Moderately/heavily exposed at enrollment= 30 Low/unexposed at enrollment= 27 Moderately/heavily exposed at 3rd trimester= 13 Low/unexposed at 3rd trimester= 8
Infants	
Enrolled	Female = 8 Male = 3 Total = 11 (goal 180)
Dysmorphology exams	Completed exams = 4 (goal 180)
2 mL blood samples collected	New infants moderately/heavily exposed= 0 New infants low/unexposed= 0 School age 7-10 year olds moderately/heavily exposed = 14 School age 7-10 year olds low/unexposed = 18
Neurobehavioral testing	6 mo. new infants moderately/heavily exposed = 0 6 mo. new infants low/unexposed = 0 12 mo. new infants moderately/heavily exposed = 0 12 mo. new infants low/unexposed = 0 School age 7-10 year olds moderately/heavily exposed = 23 School age 7-10 year olds low/unexposed = 38
Infant COR	6 mo. new infants moderately/heavily exposed = 0 6 mo. new infants low/unexposed = 0 12 mo. new infants moderately/heavily exposed = 0 12 mo. new infants low/unexposed = 0
Archived Data (Aim 2)	
Mothers	
Enrolled	Moderately/heavily exposed = 367 Low/unexposed = 358
2D ultrasound	Moderately/heavily exposed = 307 Low/unexposed= 354
Blood samples collected	Moderately/heavily exposed women enrollment= 334 Low/unexposed women enrollment= 352 Moderately/heavily exposed women 3rd trimester= 207 Low/unexposed women 3rd trimester= 265
Infants/Children	
Enrolled	Moderately/heavily exposed = 359 Low/unexposed = 360
Dysmorphology exams	6 mo. old infants Moderately/heavily exposed = 224 6 mo. old infants Low/unexposed = 256 12 mo. old infants Moderately/heavily exposed = 232 12 mo. old infants Low/unexposed = 264
Neurobehavioral testing	6 mo. old infants moderately/heavily exposed = 217 6 mo. old infants low/unexposed = 250 12 mo. old infants moderately/heavily exposed = 225 12 mo. old infants low/unexposed = 258

Category	N
	Preschool moderately/heavily exposed = 109 Preschool low/unexposed = 162
Blood samples collected	Preschool moderately/heavily exposed= 41 Preschool low/unexposed= 79
Infant COR	6 mo. old infants Moderately/heavily exposed = 138 6 mo. old infants Low/unexposed = 153 12 mo. old infants Moderately/heavily exposed = 146 12 mo. old infants Low/unexposed = 163
CIFASD Shared Data (Aim 3)	
U01 PI Mattson	CIFASD data sent = yes CoFASP data sent = yes
U01 PI Weinberg	New mother samples sent Moderately/heavily exposed = 0 Low/unexposed = 0 New infant samples sent Moderately/heavily exposed = 0 Low/unexposed = 0
U01 PI Suttie	2D ultrasound = 3 samples sent to Noble
UH2 PI Torii	Infant blood samples sent Moderately/heavily exposed new infants= 0 Low/unexposed exposed new infants = 0 Child blood samples sent Moderately/heavily exposed children 7-10 years of age = 0 Low/unexposed exposed children 7-10 years of age = 0 FASD Research Repository -= 3

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED? A postdoctoral scholar from the lab of Dr. Joanne Weinberg has been involved in activities supported by this project and has obtained opportunities for training and professional development. In addition, in Dr. Miranda's lab, Nihal Salem, a pre-doctoral student, has been working with the Ukraine data, and MD/PhD student Alexander Tseng been developing expertise with these data and has been using this project as the basis for his F31 project.

Describe your project's interrelation with aims of the CIFASD consortium its other projects and the progress on the interactions.

Collaborations with others in the CIFASD consortium included:

1. Weinberg U01 - we have collected blood samples (78 maternal and 32 child) that will be shipped from Ukraine in March, 2019, and aliquots sent to Dr. Weinberg for Markers of Inflammation. We have collected 3 blood samples and associated Health Questionnaire data from children with FASD for Dr. Weinberg from the FASD Research Repository in San Diego and are contacting an additional 37 families eligible for this sampling.
2. Torii UH2 – we have collected 3 blood samples from children with FASD from the FASD Research Repository in San Diego and shipped these, same day, to Dr. Torii.
3. Suttie U01 – 3D camera at one site will capture images of school age children being tested.
4. Noble U01 – 2D ultrasounds have been captured for at least one time point for 56 newly enrolled pregnant women in the cohort. Three sample recordings have been sent to Dr. Noble for review.
5. Mattson U01– data shared with Dr. Mattson who is using these data to test and modify Decision Tree.

Inclusion Enrollment Report

Inclusion Data Record (IDR) #: 173947

Using an Existing Dataset or Resource: No

Delayed Onset Study ?: No

Clinical Trial: No

Enrollment Location: Foreign

NIH Defined Phase III Clinical Trial: No

Study Title: Early Predictors of FASD in Ukraine - Mothers

Planned Enrollment

Comments: New mothers to be recruited in Phase IV Ukraine

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/Alaska Native	0	0		0	0					**0**
Asian	0	0		0	0					**0**
Native Hawaiian or Other Pacific Islander	0	0		0	0					**0**
Black or African American	0	0		0	0					**0**
White	200	0		0	0					**200**
More than One Race	0	0		0	0					**0**
Unknown or Not Reported										
Total	**200**	**0**		**0**	**0**					**200**

Cumulative Enrollment

Comments: New mothers recruited in Phase IV Ukraine

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/Alaska Native	0	0	0	0	0	0	0	0	0	**0**
Asian	0	0	0	0	0	0	0	0	0	**0**
Native Hawaiian or Other Pacific Islander	0	0	0	0	0	0	0	0	0	**0**
Black or African American	0	0	0	0	0	0	0	0	0	**0**
White	0	0	0	0	0	0	0	0	0	**0**
More than One Race	0	0	0	0	0	0	0	0	0	**0**
Unknown or Not Reported	0	0	0	0	0	0	0	0	0	**0**
Total	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Inclusion Data Record (IDR) #: 173948

Using an Existing Dataset or Resource: No

Delayed Onset Study ?: No

Clinical Trial: No

Enrollment Location: Foreign

NIH Defined Phase III Clinical Trial: No

Study Title: Early Predictors of FASD in Ukraine - Infants

Planned Enrollment

Comments: New live born infants to be recruited in CIFASD Phase IV Ukraine

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/Alaska Native	0	0		0	0					**0**
Asian	0	0		0	0					**0**
Native Hawaiian or Other Pacific Islander	0	0		0	0					**0**
Black or African American	0	0		0	0					**0**
White	90	90		0	0					**180**
More than One Race	0	0		0	0					**0**
Unknown or Not Reported										
Total	**90**	**90**		**0**	**0**					**180**

Cumulative Enrollment

Comments: New live born infants recruited in CIFASD Phase IV Ukraine

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/Alaska Native	0	0	0	0	0	0	0	0	0	**0**
Asian	0	0	0	0	0	0	0	0	0	**0**
Native Hawaiian or Other Pacific Islander	0	0	0	0	0	0	0	0	0	**0**
Black or African American	0	0	0	0	0	0	0	0	0	**0**
White	0	0	0	0	0	0	0	0	0	**0**
More than One Race	0	0	0	0	0	0	0	0	0	**0**
Unknown or Not Reported	0	0	0	0	0	0	0	0	0	**0**
Total	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

Inclusion Data Record (IDR) #: 173949

Using an Existing Dataset or Resource: Yes

Delayed Onset Study ?: No

Clinical Trial: No

Enrollment Location: Domestic

NIH Defined Phase III Clinical Trial: No

Study Title: Early Predictors of FASD in Ukraine - CIFASD

Planned Enrollment

This study involves an existing dataset and does not include planned enrollment.

Cumulative Enrollment

Comments: Archived data from Phase II and III CIFASD U01 Mattson

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/Alaska Native	4	1	0	2	2	0	1	0	0	**10**
Asian	2	3	0	0	0	0	0	0	0	**5**
Native Hawaiian or Other Pacific Islander	0	0	0	0	0	0	0	0	0	**0**
Black or African American	15	9	0	1	0	0	0	1	0	**26**
White	56	82	0	11	7	0	1	1	0	**158**
More than One Race	19	17	0	2	3	0	1	0	0	**42**
Unknown or Not Reported	0	0	0	0	2	0	0	0	0	**2**
Total	**96**	**112**	**0**	**16**	**14**	**0**	**3**	**2**	**0**	**243**

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	Sowell KD, Uriu-Adams JY, Van de Water J, Chambers CD, Coles CD, Kable JA, Yevtushok L, Zymak-Zakutnya N, Wertelecki W, Keen CL. Implications of altered maternal cytokine concentrations on infant outcomes in children with prenatal alcohol exposure. Alcohol (Fayetteville, N.Y.). 2018 May;68:49-58. PubMed PMID: 29453023; PubMed Central PMCID: PMC5820219.
Complete	Bodnar TS, Raineki C, Wertelecki W, Yevtushok L, Plotka L, Zymak-Zakutnya N, Honerkamp-Smith G, Wells A, Rolland M, Woodward TS, Coles CD, Kable JA, Chambers CD, Weinberg J. Altered maternal immune networks are associated with adverse child neurodevelopment: Impact of alcohol consumption during pregnancy. Brain, behavior, and immunity. 2018 October;73:205-215. PubMed PMID: 29738852; PubMed Central PMCID: PMC6344127.
In Process at NIHMS	Coles CD, Kable JA, Granovska IV, Pashtepa AO, Plotka LD, Dolhov VB, Wertelecki W, Jones KL, Chambers CD. Gestational age and socioeconomic status as mediators for the impact of prenatal alcohol exposure on development at 6 months. Birth defects research. 2018 October 31. PubMed PMID: 30378744.
Complete	Bandoli G, Coles CD, Kable JA, Wertelecki W, Yevtushok L, Zymak-Zakutnya N, Wells A, Granovska IV, Pashtepa AO, Chambers CD. Patterns of Prenatal Alcohol Use That Predict Infant Growth and Development. Pediatrics. 2019 January 4. PubMed PMID: 30610099; PubMed Central PMCID: PMC6361345.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Nothing to report

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? No

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization?

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

*Publications [In Preparation & Submitted]

1.Xu R, Wells A, Chambers CD. Growth measurements together with maternal characteristics predict infant development in a prenatal alcohol exposed cohort. Submitted.

2. Sowell KD, Uriu-Adams JY, Chambers CD, Coles CD, Kable JA, Holt RR, Yevtushok L, Zymak-Zakutnya N, Wertelecki W, Keen CL and the CIFASD. Altered plasma fatty acid composition in Ukrainian women consuming alcohol during pregnancy and its association with Fetal Alcohol Spectrum Disorder. Submitted.

*Poster Abstracts and Presentations

Abstracts

1. Bodnar TS, Raineki C, Wertelecki W, Yevtushok L, Plotka L, Zymak-Zakutnya N, Well A, Honerkamp-Smith G, Coles CD, Kable JA, Chambers CD, Weinberg J, the CIFASD. Cytokine disturbances associated with prenatal alcohol exposure in children: implications for health and development. Alcohol Clin Exp Res. 2018;42(S1):46A. Presented at the 41st Annual Scientific Meeting of the Research Society on Alcoholism. San Diego, CA, June 2018. (Poster)

2. Doyle LR, Chambers CD, Jones KL, Mattson SN, the CIFASD. Validation of a decision tree for clinical identification of children affected by prenatal alcohol exposure in a low-risk sample. Alcohol Clin Exp Res. 2018;42(S1):115A. Presented at the 41st Annual Scientific Meeting of the Research Society on Alcoholism. San Diego, CA, June 2018. (Poster)

Presentations

1. Chambers CD. “Nutritional interventions for fetal alcohol spectrum disorders.” 3rd International ENTIS OTIS; September 4-8, 2018, Newcastle upon Tyne, United Kingdom.

2. Chambers CD. CIFASD longitudinal cohort study. Presented at Lutsk Regional Children’s Hospital and University, Lutsk, Volyn Oblast, Ukraine, September 2018.

3. Coles CD and the CIFASD, Neurodevelopmental outcomes of prenatal alcohol exposure: Experience of the Ukrainian cohort. Presented at Lutsk Regional Children’s Hospital and University. Lutsk, Volyn Oblast, Ukraine, September 2018.

Symposia

1. Miranda RC; Tseng A, Salem N, Mahnke A, Allan A, Wells A, Chambers C. MicroRNA biomarkers and mediators of prenatal alcohol effects. Alcoholism Clinical and Experimental Research. 2018; 42(Supplement 2):abstract 265. Presented at the 19th

Congress of International Society for Biomedical Research on Alcoholism (ISBRA 2018). Tokyo, Japan, September 13, 2018.

2. Coles CD, Kable JA, Mesa DA, Coleman TP, Jones KL, Yevtushok L, Kulikovsky Y, Wertelecki W, Chambers CD. Early identification of effects of prenatal alcohol exposure: infant cardiac orienting response as a biomarker. Alcohol Clin Exp Res. 2018;42(S1). Presented at the 41st Annual Scientific Meeting of the Research Society on Alcoholism. San Diego, CA, June 2018.

3. Tseng AM, Balaraman S, Allan AM, Chambers C, Miranda RC, and the CIFASD. Endocrine MiRNAs in pregnant women, predictive of FASD infant outcomes, control placental trophoblast growth, survival, and maturation. Alcohol Clin Exp Res. 2018;42(S1). Presented at the 41st Annual Scientific Meeting of the Research Society on Alcoholism. San Diego, CA, June 2018.

4. Bodnar TS, Raineki C, Wertelecki W, Yevtushok L, Plotka L, Zymak-Zakutnya N, Honerkamp-Smith G, Wells A, Rolland M, Woodward TS, Coles CD, Kable JA, Chambers CD. Weinberg J, and the CIFASD. Alcohol intake and immune function: associations between maternal immune networks and child neurodevelopmental outcome. Alcohol Clin Exp Res. 2018;42(S1):247A. Presented at the 41st Annual Scientific Meeting of the Research Society on Alcoholism. San Diego, CA, June 2018.

5. Suttie M, Wetherill L, Jacobson SW, Jacobson JL, Hoyme EH, Mattson S, Sowell ER, Coles C, Wozniak JR, Riley EP, Jones KL, Mukherjee R, Foroud T, Hammond P. Using 3D facial analysis to identify minor facial anomalies and ethnic differences in effects of prenatal alcohol exposure. Alcohol Clin Exp Res. 2018;42(S1):247A. Presented at the 41st Annual Scientific Meeting of the Research Society on Alcoholism. San Diego, CA, June 2018. (Symposium)

1. Chambers CD, Wells A, Xu R, Wertelecki W, Coles, CD, Kable J, Zymak-Zakutnya, Yevtushok L. A growth modeling approach to predicting future neurodevelopmental performances in infants with prenatal alcohol exposure. Alcohol Clin Exp Res. 2018;42(S1):247A. Presented at the 41st Annual Scientific Meeting of the Research Society on Alcoholism. San Diego, CA, June 2018. (Symposium)

2. Kable J, Coles C, Yevtushok L, Zymak-Zakutnya N, Wertelecki W, Jones K, Chambers C. The impact of micronutrient supplementation in alcohol-exposed pregnancies on reaction time responses in Ukrainian preschoolers. 8th International Conference on Fetal Alcohol Spectrum Disorders, Vancouver, BC, Canada, March 2019 (Symposium)

3. Chambers CD, Wells A. The role of trajectories in clinical prediction of FASD outcomes. 8th International Conference on Fetal Alcohol Spectrum Disorders, Vancouver, BC, Canada, March 2019 (Symposium)

Chambers U01 Ukraine	Current Month	Cumulative Total	May 2019 Goal	% to Goal	Overall Goal	Completion Goal Date	May 2018 Goal	May 2019 Goal	May 2020 Goal	May 2021 Goal	May 2022 Goal
Newly Recruited Mothers											
Exposed mothers	5	30	60	50.0%	120	5/31/2020	10	60	110	120	120
Low/unexposed mothers	1	29	41	70.7%	80	5/31/2020	6	41	76	80	80
Neurobehavioral Testing											
School age exposed	5	23	27	85.2%	80	5/31/2021	0	27	54	80	80
School age low/unexposed	3	38	24	158.3%	70	5/31/2021	0	24	47	70	70
6 mo. old infants exposed	0	0	8	0.0%	60	5/31/2021	0	8	34	60	60
6 mo. old infants low/unexposed	0	0	4	0.0%	40	5/31/2021	0	4	22	40	40
12 mo. old infants exposed	YR3	YR3	0	YR3	60	1/1/2022	0	0	8	34	60
12 mo. old infants low/unexposed	YR3	YR3	0	YR3	40	1/1/2022	0	0	4	22	40
Blood Samples Collected											
Infants exposed	0	0	10	0.0%	80	5/31/2021	0	10	45	80	80
Infants low/unexposed	0	0	8	0.0%	60	5/31/2021	0	8	34	60	60
School age children exposed	0	14	13	107.7%	40	5/31/2021	0	13	26	40	40
School age children low/unexposed	0	18	10	180.0%	30	5/31/2021	0	10	20	30	30
2D Ultrasounds											
Exposed mothers	15	45	60	75.0%	120	5/31/2020	10	60	110	120	120
Low/unexposed mothers	10	45	41	109.8%	80	5/31/2020	6	41	76	80	80
3D Images											
Exposed school age children	5	9	12	75.0%	35	5/31/2021	0	12	24	35	35
Low/unexposed school age children	3	11	10	110.0%	30	5/31/2021	0	10	20	30	30

	Start	End
Current month (defined by project) =	1/1/2019	1/31/2019
Date of project numbers update entry =	2/5/2019	

Cumulative - at the end of each year.

Recruitment through 12/1/18

Sample	N Collected	Goal 5/31/2019	% of Goal	Overall Goal	Date to Complete
Pregnant Women Enrolled	50	101	50%	200	5/31/20
Neuro Testing 7-10 Year Olds	47	51	92%	150	5/31/21
Neuro Testing Infants 6-12 Months	0	12	0	100 each	1/1/22
Blood Samples Pregnant Women	50	101	50%	200	5/31/20
Blood Samples Infants	1	18	6%	140	5/31/21
Blood Samples 7-10 Year Olds	20	23	87%	70	5/31/21
2D Ultrasounds	50	101	50%	200	5/31/20
3D Images	12	22	55%	65	5/31/21

Progress

- 5 papers published/accepted in 2018
  - Additional 2 under review and 5 in preparation

1. Coles et al Birth Defects Res 2018. Gestational age and socioeconomic status as mediators for the impact of prenatal alcohol exposure on development at 6 months.
2. Bodnar et al Brain Behav Immun 2018. Altered maternal immune networks are associated with adverse child neurodevelopment: impact of alcohol consumption during pregnancy.
3. Chan et al. Comun Stat Simul Comput 2018. A study of R^2 measure under the accelerated failure time models.
4. Sowell et al Alcohol 2018. Implications of altered maternal cytokine concentrations on infant outcomes in children with prenatal alcohol exposure.
5. Bandoli et al Pediatrics 2018 in press. Patterns of prenatal alcohol use that predict infant growth and development.

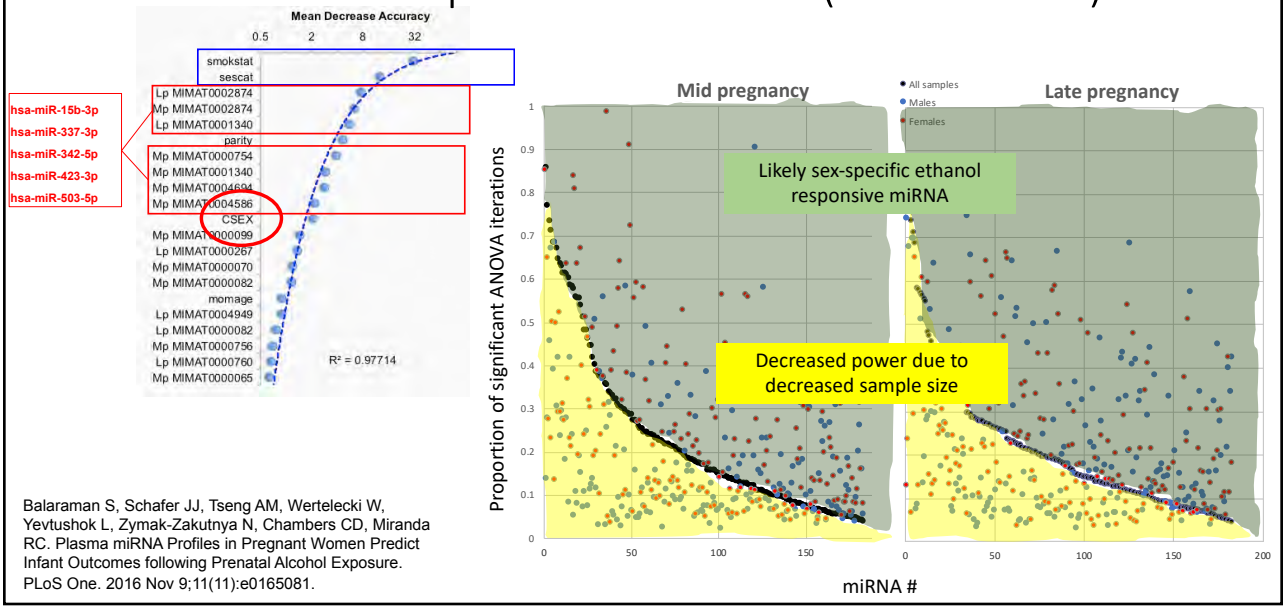
- Shipped first blood sample from FASD child to Torii's December, 2018
- Awarded R01 to add fNIRS and develop wireless point of care screening on cardiac orienting response
- Working with Sarah to develop algorithm to apply to retrospective sample, and shared data to test/modify Decision Tree
- Sent clinical variables to Mike Suttie for all 3D image analyses

Progress 2018

CIFASD

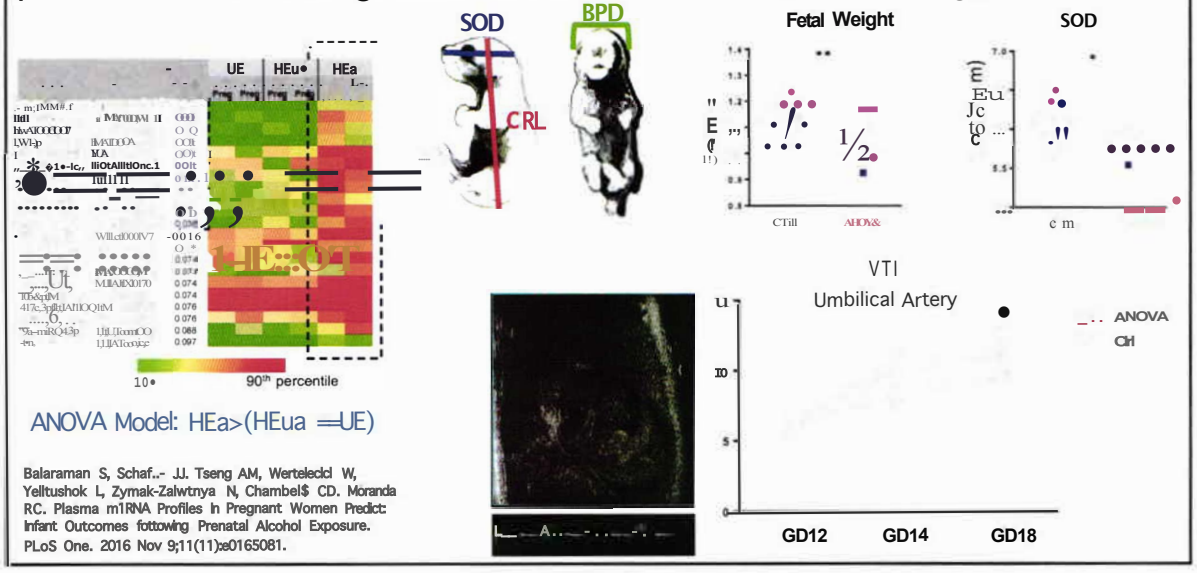
Miranda Group (Amanda Mahnke, Nihal Salem, Alex Tseng) in collaboration with Christina Chambers

Bootstrap-resampling to estimate impact of infant sex on maternal miRNA response to Ethanol (Nihal Salem)



Balaraman S, Schafer JJ, Tseng AM, Wertelecki W, Yevtushok L, Zymak-Zakutnya N, Chambers CD, Miranda RC. Plasma miRNA Profiles in Pregnant Women Predict Infant Outcomes following Prenatal Alcohol Exposure. PLoS One. 2016 Nov 9;11(11):e0165081.

Maternal miRNAs, elevated in the HEa group result in decreased placental and fetal growth, and blood flow (Alex Tseng)



Balaraman S, Schaf..- JJ. Tseng AM, Wertelecld W, Yelltushok L, Zymak-Zalwtnya N, Chambel$ CD. Moranda RC. Plasma m1RNA Profiles In Pregnant Women Predict: Infant Outcomes fottowing Prenatal Alcohol Exposure. PLoS One. 2016 Nov 9;11(11):e0165081.

Progress 2018

- Nihal Salem: Tim Cudd Research Award FASDSG 2018
- Alex Tseng: Enoch Gordis Research Award RSA 2018
- Amanda Mahnke:
  - Maternal samples - 25 samples which overlap with Joanne's cytokine data
  - Child Samples - 29 samples+ an additional 9 which overlap with Joanne's (19 remaining)= 38 total child samples
  - Overcame heparinase inhibition in child samples collected in heparin-coated tubes.
- Alex's paper has been submitted for review, and has been uploaded to BioArchives
- Nihal is redoing analysis with additional maternal samples. Plans to write up the paper in 2019

Progress Report: March 2019 [eRA Commons issue; submitting via Word]

Investigator(s): Claire D. Coles; Therese Grant (Subcontract)

Institution(s): Emory University School of Medicine; University of Washington School of Medicine (Subcontract)

CIFASD4 Project Title: Fetal Alcohol Spectrum Disorders in Adults: Health and Neurobehavior

Grant Number: U01AA026108

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

1. Establish a Registry of individuals with prenatal alcohol exposure (PAE) who are willing to participate in future research, beginning with those who are enrolled in the large Seattle and Atlanta studies and survey their health status. The project will target 500 individuals who will include individuals older than 30 years of age with prenatal exposure or FASD diagnosis as well as unexposed contrast groups. Tier 1 assessment (N=500; 250 per site) will include a demographic and adult health survey.

2. In a subsample of adults selected from these two cohorts (N=240; 120 per site), evaluate in depth current status in areas supporting adult physical wellbeing and social functioning, including dysmorphology, mental health, substance use and aspects of adaptive and social functioning where deficits may not have been apparent earlier in life. In addition, this Tier 2 assessment will confirm self-reported health information through medical records abstraction. These data will allow a description of the changing phenotypes of FASD with age as well as the adaptive and social functioning of affected adults in order to evaluate the following hypotheses:

H1) The physiological and behavioral characteristics of alcohol-affected adults (older than 30 years) are significantly different from those of unexposed contrast groups and a pattern of physiology and behavior will be evident in adults with FASD that can be used to modify diagnostic criteria for adults which may differ from those most effective in children.

H2) Adults with PAE will have a greater degree of adaptive and social dysfunction than unexposed controls when socioeconomic status is controlled.

H3) Health problems will be found to be associated with PAE. These will include problems in the following areas: Cardiovascular, vision, metabolic/endocrine (diabetes, thyroid), mental health (specifically anxiety, depression and substance abuse), and immune/autoimmune function.

3. This goal involves collaboration with Dr. Weinberg's CIFASD project and will assess immunological status of alcohol exposed and affected adults. We will relate these findings from the immune studies to health status and behavioral measures collected in Goal 2. Blood samples will be collected in Tier 2 and sent to Dr. Weinberg's lab in Vancouver for analysis. We will collaborate on analysis and reporting of outcomes.

H4) PAE history will be associated with persistent alterations/dysregulations in immune functioning that will be related to physical and mental health, providing a window into factors underlying risk and resilience for adults with PAE and FASD diagnosis.

B.1.a Have the major goals changed since the initial competing award or previous report? No.

B.2 What was accomplished under these goals?

1) Major Activities. In 2019 we continued to refine and test the methodology and instruments for these studies as well as initiated data collection.

a. **Cross-Site Collaboration**. An important accomplishment was the establishment of collaboration between sites and with Dr. Weinberg's lab. This was accomplished through frequent contact as well as, initially, weekly inter-site conference calls among the 3 sites. More recently, this has been reduced to once a month as most issues have been successfully resolved.

b. **Initiation of Tier 2 Data Collection.** While Tier 1 recruitment has continued, we began data collection for Tier 2 in November/December of 2018. Refinement of instruments and piloting of data collection procedures for the Tier 2 Protocol occupied much of this year. It was important both to establish protocols that could be carried out in different sites and to assure reliability of measurement among sites. As of this writing, Seattle has completed 15 Tier 2 evaluations and Atlanta has completed 16. Our data collection rate is consistent with our timeline. The data collection protocols have been carried out without problems.

c. **Data Management**. Develop data collection and storage protocols and methods. Atlanta staff, with the consultation with Dr. Grant, Dr. Radin and Seattle staff, developed data collection and storage instruments using REDCap facilities at Emory that can be directly linked to the Seattle site. These were created, evaluated and modified over this year and placed into "active" status in January 2019 allowing direct entry of information in to these databases. Data dictionaries and sample de-identified data from these programs have been provided to the Indiana Informatics Core to allow storage of CIFASD data and eventual data sharing.

d. **Additional Studies initiated.** Two "associated" studies were funded that rely on this CIFASD longitudinal study.

- In Atlanta, a R21 was funded with Julie A Kable, PhD as the Principle Investigator that is collecting data on micro vascularization and cardiac health as well as frontal lobe functioning in 80 adults participating in Tier 2. This study is actively underway with the collaboration of Dr. Coles and Puja K. Mehta, MD, Director of Women's Translation Cardiovascular Research of the Department of Cardiology, as Co-Investigators.
- In Seattle, Drs. Grant and Radin are collaborating with Eileen Moore, PhD (PI, San Diego State University) on her R01 project "Brain Maturation in Adults with FASD." This R01 will recruit 90 diagnosed individuals and controls who 1) had previous structural MRI scans done in our earlier UW studies; and 2) are now completing neurobehavioral testing as part of Phase IV CIFASD. These 90 individuals will have another MRI session in which structural, DTI, and connectivity assessments will be conducted. Comparisons between these and earlier scans will provide insight into the changes in overall brain structure, white matter integrity, and function with age. Startup of the R01 has been delayed at the UW because of MRI scanner upgrades being done. We now estimate starting scans in Spring 2019.

2) **Resolution of Data Breach in Seattle**. In November 2017 Dr. Grant submitted a report to the University of Washington (UW) Institutional Review Board (IRB) involving a study staff person who, against all regulations and protocols, took a portable "thumb drive" home containing confidential, personal information regarding 433 former study subjects. The thumb drive was subsequently lost. The UW IRB reviewed the event and in May 2018 required the researchers to take the following corrective actions: Notify all 433 participants of the possible data breach before any recruitment effort was made into the next study, using separate scripts and/or letters for two groups: 1) The 175 participants whose names were associated with identifiable private information were to be immediately informed by phone or letter; b) The

remaining 258 participants, whose names were associated with only a study identifier, were to be informed by phone or letter as they were recruited for the next study.
Upon notification of these events, the NIAAA requested a teleconference to discuss their concerns. The teleconference occurred on June 11, 2018 and included representatives from NIAAA, OHRP, UW Office of Sponsored Programs, UW Institutional Privacy Official, and Human Subjects Division as well as the UW PI and the lead site PI at Emory University. Notification to all 175 participants whose names were associated with identifiable private information had been completed at this time. In response to the NIAAA feedback on further corrective actions steps to take, the UW researchers immediately sent out letters to the remaining 258 participants, and UW IRB informed NIAAA OHP that the required corrective action had been completed. On November 26, 2018 UW IRB received the following from NIAAA OHRP: "The Office for Human Research Protections (OHRP) has received your letter of June 14, 2018, concerning the above protocol, which was submitted to fulfill the requirements of reporting to OHRP described in the Department of Health and Human Services (HHS) regulations at 45 CFR 46.103(a) and 46.103(b)(5). Based on the information provided to us, your report and the corrective actions described appear to be appropriate under HHS regulations and your institution's Assurance of Compliance."

2) Specific Objectives.

- Establish a Registry: This has been done at both sites and data combined in REDCap. We are at 75% of the May 2019 goal as of February, 2019.
    - In Seattle, 71 Health Surveys have been collected. Three individuals refused participation. The study continues to use a variety of search activities to identify and recruit participants.
    - In Atlanta, 47 individuals have completed the Health Survey and agreed to the registry. No individuals have withdrawn from the study or refused participation.
- Tier 2: In–Depth Testing. Selected individuals are being recruited and seen for in-depth testing of current status. As of February 22, 2019, 31 individuals at both sites had completed assessment (65% of yearly goal). As we began collection in December of 2018 for Tier 2, this rate is encouraging.
- Developed data collection and storage instruments using REDCap. One of several advantages of this method is that both Atlanta and Seattle can use the same instruments and still maintain group/site identity. In addition, the same instruments were provided to the Informatics Core in Indiana and will allow efficient and timely transfer of data to the data repository there.
- Blood samples have been collected and sent to Vancouver for analysis without problems.
- 2-D photographs have been taken and sent to Dr. Suttie without problems.
- Saliva samples have been collected and sent to Indiana for genetics studies.
- Dysmorphology is being done by Margaret Adams, MD, in Seattle at the time of the Tier 2 assessment. It will be carried out in Atlanta by Dr. Jones using a group format later in 2019.

3) Significant Results.

It is early in the process for specific results. We have noted the following during the process of recruitment and data collection.

- In Seattle, during online searches for participants, it was found that three times as many FAS/FAE diagnosed former subjects (23/438, or 5.25%) had died (mean age at death 36.8 years) compared to control subjects (1/60, or 1.67%) (Age 35 years). To examine this issue further, death certificates were obtained for 18 individuals. Causes of death among the diagnosed include physical health conditions: heart-related (27.8%), cancer (16.7%), kidney-related (5.6%), liver-related (5.6%), diabetes (5.6%); and conditions related to mental health: alcohol and/or drug abuse (16.7%), accidents (11.1%), suicide (5.6%), and violence (5.6%). Cause of death for the control was cancer.
- In Atlanta, during the initial outreach wave (N=234), 9 male individuals have been found to be incarcerated in Federal Prison. Using the databases available through the Georgia Department of Corrections, information about these individuals has been obtained. Two are expected to be released during the grant period and will be followed then. Others have longer terms expected. Given these findings, we will use the same data bases to search for any individuals who have been lost to follow-up or unresponsive.
- In Seattle during initial outreach (N=438), two male individuals were confirmed incarcerated and both will be released in time to be recruited for the study. One additional male is believed to be incarcerated based on a news article. All three of these individuals have FAE diagnoses.
- In Atlanta, 16 participants have completed the Tier 2 data collection protocol. Of these, six were from the control group and 10 were alcohol-exposed. Two participants (both alcohol-exposed) had out-of-range results on the gamma glutamyl transferase (GGT) test, a measure of a liver enzyme sensitive to excessive alcohol use. For the drug screen results, nine were positive for marijuana and three of these also were positive for cocaine. Six of the nine positive marijuana results and two of the three positive cocaine results were from alcohol-exposed participants.
- In Seattle, 15 participants have completed the Tier 2 data collection protocol. Of these, 4 were in the FAS group, 8 were in the FAE group, and 3 were controls. All participants' GGT test results were "in range". Five participants tested positive for marijuana metabolites (1 FAS, 2 FAE, 2 controls). Other than marijuana, no participant tested positive for any other substance.
- In Atlanta, as part of Dr. Kable's supplemental study, of the initial 8 subjects enrolled in Tier 2, the results of the physical examination and diagnostic blood work resulted in four participants needing to be referred to address medically urgent outcomes. Within the four participants, 2 were anemic, 3 had high cholesterol (high LDL), 1 had clinically urgent elevated blood pressure levels and 1 was identified as being pre-diabetic. Community resources regarding low income and indigent care were provided to facilitate seeking medical consultation for those who did not have an existing health-care provider. Other medical problems were noted that did not require urgent care and participants have been notified by mail.

4) Key Outcomes and Other Achievements. Currently, outcomes are process based or related to the development of instruments.

B.3 Competitive Revisions/Administrative Supplements. For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required? No.

B.4 What opportunities for training and professional development has the project provided? We have provided post-doctoral fellows and graduate and undergraduate students in psychology the opportunity to work on the project

B.5 How have the results been disseminated to communities of interest? No results to disseminate at this time as data collection is on-going.

B.6 What do you plan to do during the next reporting period to accomplish the goals? During the next 4 months, we will continue recruitment and data collection for both Tier 1 and Tier 2 in both in Atlanta and in Seattle. At this time, we do not plan to vary from the approved study design. Any changes will be the result of observations of the impact of the current plans.

***Publications [Accepted & In Press]. Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph, or preprint) during the reporting period resulting directly from this award?** Not as yet.

***Publications [In Preparation & Submitted]** None from this project as yet

***Poster Abstracts and Presentations**

Coles, CD, Grant, T, Weinberg, J. Mapping the Undiscovered Country: Physical and Mental Health in Adults with FASD. Presented at the 8th International Research Conference on Adolescents and Adults with FASD: Review, Respond and Relate: Integrating Research, Policy and Practice around the World, Vancouver, BC, April 18-21, 2018.

Under the supervision of Dr. Grant, University of Washington Psychology student intern, Kristina Rowlett, is planning to present an abstract on mortality in the FASD longitudinal sample at the 2019 UW Undergraduate Research Symposium.

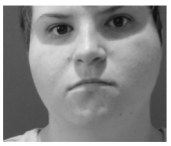
Describe your project's interrelation with aims of the CIFASD consortium its other projects and the progress on the interactions. This project extends CIFASD's investigation of FASD into middle adulthood as the first study to describe the impact of prenatal alcohol exposure on adult functioning in longitudinal samples of adults whose prenatal exposure can be documented or who were diagnosed as children. In order to carry out this project, we collaborate both between Atlanta and Seattle and with several other CIFASD projects including the Dysmorphology core, under Dr. Jones, which will identify physical findings in adults. To carry out the immunological goals of the study, we are working closely with Dr. Weinberg in Vancouver who is analyzing samples that we provide to her while carrying out a parallel study of adults in Canada using the same protocol and measures. This collaboration increases our sample size and generalizability substantially. Working with Michael Suttie, we are sending 2-D photographs of adults taken in all 3 sites that will be analyzed for features of FASD. Finally, we are working with the Informatics Core to record and archive these data using a REDCap format.

Coles U01 Adults	Current Month	Cumulative Total	May 2019 Goal	% to Goal	Overall Goal	Completion Goal Date	May 2018 Goal	May 2019 Goal	May 2020 Goal	May 2021 Goal	May 2022 Goal
Enrollment											
Number of adult registry enrollees (Atlanta and Seattle)	2	118	153	77%	500	5/31/2022	33	153	273	393	500
Questionnaires - Demographics and Health											
ATL Qs completed - FASD or PAE	0	31	50	62%	166	5/31/2022	12	50	91	131	166
ATL Qs completed - CON	0	16	25	64%	83	5/31/2022	5	25	45	65	84
SEA Qs completed - FAS	1	29	26	112%	84	5/31/2022	6	26	46	66	83
SEA Qs completed - FAE	1	25	26	96%	84	5/31/2022	6	26	46	66	84
SEA Qs completed - CON	1	17	25	68%	83	5/31/2022	4	25	45	65	83
Questionnaires - Total	*3*	*118*	*152*	*78%*	*500*	*5/31/2022*	*33*	*152*	*273*	*393*	*500*
Biosamples and Neurobehavioral Testing (NIH Tool Box and Qs)											
ATL Biosamples and NB - FASD	2	6	8	75%	40	1/1/2022	0	8	20	32	40
ATL Biosamples and NB - PAE	0	4	8	50%	40	1/1/2022	0	8	20	32	40
ATL Biosamples and NB - CON	4	6	8	75%	40	1/1/2022	0	8	20	32	40
SEA Biosamples and NB - FAS	1	4	8	50%	40	1/1/2022	0	8	20	32	40
SEA Biosamples and NB - FAE	1	8	8	100%	40	1/1/2022	0	8	20	32	40
SEA Biosamples and NB - CON	0	3	8	38%	40	1/1/2022	0	8	20	32	40
Biosamples and NB Testing Total	*8*	*31*	*48*	*65%*	*240*	*1/1/2022*	*0*	*48*	*120*	*192*	*240*

Cumulative Goal at the end of each year.

	Start	End
Current month (defined by project) =	1/19/2019	2/22/2019
Date of project numbers update entry =	2/22/2019	

Mid Year Progress: FY 2019
Fetal Alcohol Spectrum Disorders in Adults: Health and Neurobehavior: Atlanta and Seattle



Claire D. Coles, PhD, Therese Grant, PhD, Edward P. Riley, PhD,
in collaboration with Joanne Weinberg, PhD

- Data Collection
  - Tier 1-Health Survey-Current total N=110, 73% of Yearly goal
  - Tier 2-Biosamples and Neurodevelopment, N=20, 42% of Yearly goal.
- Data Management
  - Created 90% of databases in RedCap
  - Provided databases to Indiana
  - Currently entering data into databases
- Site Collaboration
  - Regular collaboration with Seattle and Vancouver, weekly calls
  - Blood samples sent to Vancouver from Seattle and Atlanta

Opportunities and Challenges?

- Integration of new grants
  - Seattle-Brain Maturation in Adults with FASD, PI: Eileen Moore, Ph.D. (San Diego State University)
  - Atlanta-Cardiovascular Risk in Adult FASD and its Impact on Prefrontal Cortical Functioning, PI: Julie A. Kable, PhD (Emory University)

- Location of subjects and recruitment
  - Prisoner issue? Should we try to recruit subjects who are incarcerated?

- Facial features and photographs.
  - Method identified for transferring 2-D photographs

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The major goals of this study are to:

1.Develop a web portal with a novel, online consenting process to create a large CIFASD cohort for new studies.
a.Develop the ability to upload 2D facial images and complete neurobehavioral assessments online.
b.Implement the collection of saliva samples for DNA isolation.
c.Facilitate the recruitment and consenting of individuals for other studies and online intervention protocols.
2.Perform whole exome sequencing in a targeted set of the newly recruited online CIFASD cohort with features consistent with a high or low probability of Fetal Alcohol Syndrome (FAS) or FASD.
a.Combine data from 2D facial images and neurobehavioral assessments collected in individuals participating in the online cohort to develop a novel risk score to quantify the likelihood that the individual has FAS or FASD.
b.Perform gene-based burden analysis of DNA sequence data to evaluate whether variants in the genes or pathways identified from animal models are also associated with risk of FAS or FASD in humans.
c.Perform genome-wide gene-based analyses to identify genes with increased (risk) or decreased (protective effect) burden of variants in high probability FAS or FASD subjects as compared with their contrast group with low likelihood of FAS or FASD.
3.Maintain a central informatics resource to manage sharing of data within CIFASD and with the broader research community.
a.Aggregate data collected by CIFASD projects and provide a uniform infrastructure to facilitate cross project collaborations with CIFASD.
b.Support the review of requests for CIFASD data from external researchers and provide de-identified data to approved researchers.
c.Maintain an online CIFASD registry to consent subjects interested in participating in future research studies.

Cohesiveness within CIFASD: This cooperative agreement has close collaborations with other projects in the consortium. This project partners closely with all resources (Administrative, Dysmorphology, Outreach/Dissemination), as well as multiple basic research projects focusing on animal models (Eberhart/Parnell, Hashimoto/Torrii). In addition, it has close ties with clinical research projects focusing on translational science (Chambers, Coles, Jones, Mattson, Hammond, Wozniak, Weinberg) and interventional studies (Petrenko/Tapparello). The Informatics Group within the Genetics Project interacts with many of the projects in CIFASD IV.

The primary change that occurred from the submitted application to the funded application was the inclusion of the primary aims of the Informatics Core into this application. This has occurred smoothly because both investigators (Foroud, Barnett) are at the same institution and the same department. Our staff have collaborated in various capacities over the years.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

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B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

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B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

With Specific Aim 1, outreach to communities of interest is a priority for this project. Although there are no results for Specific Aim 1 yet, we have undertaken several efforts to disseminate information about the project and its significance to communities of interest as part of our recruitment efforts. Our web portal (https://digfasd.org) describes the project, and also provides background information about FASD, resources for people and families with FASD, and information regarding research on FASD. Information about the study has been shared with the National Organization for Fetal Alcohol Syndrome (NOFAS) and its local affiliates, who have added this information to their websites and email newsletters. In October 2018, the PI, Dr. Foroud, gave a live webinar hosted by NOFAS about the project and its potential impact on the field. This webinar is now available to the public on the NOFAS YouTube channel and on our project web portal. In February of 2019, we met in person with our local NOFAS affiliate, Indiana NOFAS and gave a presentation on the project. We have also contacted FASD clinics and resource centers throughout the United States and Canada with information about our study. Finally, we post daily information about the project, other CIFASD projects, and research articles of interest to the FASD community to our social media accounts on Facebook and Twitter. Study information reaches our 191 social media followers directly, but we also share our posts on FASD community social media platforms as well. We anticipate that we will continue to develop these communication relationships as

the study progresses and use these channels to disseminate our results to the FASD community in the future. Over the next few months, we will broaden our scope to contact and share information with FASD organizations and clinics in other English-speaking countries such as England, Australia, and New Zealand.

With Specific Aim 2, we are continuing to share results of the WES analyses utilizing data from the previous CIFASD grant cycle with other projects within CIFASD.

With Specific Aim 3, we are finalizing the datasets available on the CIFASD website page http://cifasd.org/data-sharing/ to facilitate the process of requesting and accessing the CIFASD data from phases 1-3 for external researchers. We are also constructing a REDCap database to make current CIFASD data from all projects available to CIFASD investigators.

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Aim 1: Enrollment in this study has been lower than expected, which has impeded the progress of Aims 2 and 3. To meet our enrollment targets we will continue to test and hone our Facebook ads to optimize advertisement and enrollment. We will also begin recruitment and enrollment of participants in FAS clinics in Indiana and as part of other CIFASD projects. We will develop an online video to describe the project that is appropriate for cognitively impaired adults, which can be used for consent.

Aim 2: We have been performing additional analyses of our existing whole exome sequence data and are performing follow-up analyses of promising genes. We are sharing results with the animal and immune projects. Comparing results between these projects will enable us to identify genes and pathways of common interest in both animal and human projects. As we accrue additional samples, we will continue to generate whole exome sequencing data and will try to replicate our results.

Aim 3: We are working to develop data dictionaries for each of the CIFASD projects. The focus for the coming year is to receive quarterly data from each project. We are working with Drs. Coles, Chambers and Weinberg to create data dictionaries and matching data files to efficiently store data for maternal and infant miRNAs, cytokine profiles and epigenetic factors, and to make these data easily available to other researchers. We are assisting Drs. Mattson and Wozniak to convert existing neurobehavioral and MRI-related data into REDcap, and to create matching data dictionaries, which will also be made available to other researchers. Following approval from NIH, data from phases 1-3 can now be requested. This project will continue to manage the review of requests for CIFASD data from external researchers.

B.2 What was accomplished under these goals?

Goal	Target Completion Date	Percent Completed
1. Develop a web portal with a novel, online consenting process to create a large CIFASD cohort for new studies.		
a. Develop the ability to upload 2D facial images and complete neurobehavioral assessments online.	10/31/2018	2D Images 100%; Neuro-behavioral 0%
b. Implement the collection of saliva samples for DNA isolation.	01/31/2018	100%
c. Facilitate the recruitment and consenting of individuals for other studies and online intervention protocols.	06/30/2018	50%
2. Perform whole exome sequencing in a targeted set of the newly recruited online CIFASD cohort with features consistent with a high or low probability of Fetal Alcohol Syndrome (FAS) or FASD.		
a. Combine data from 2D facial images and neurobehavioral assessments collected in individuals participating in the online cohort to develop a novel risk score to quantify the likelihood that the individual has FAS or FASD.	01/31/2019	0%
b. Perform gene-based burden analysis of DNA sequence data to evaluate whether variants in the genes or pathways identified from animal models are also associated with risk of FAS or FASD in humans.	01/31/2019	20%
c. Perform genomewide gene-based analyses to identify genes with increased (risk) or decreased (protective effect) burden of variants in high probability FAS or FASD subjects as compared with their contrast group with low likelihood of FAS or FASD.	03/31/2019	20%
3. Maintain a central informatics resource to manage sharing of data within CIFASD and with the broader research community.		
a. Aggregate data collected by CIFASD projects and provide a uniform infrastructure to facilitate cross project collaborations with CIFASD.	03/31/2022	30%
b. Support the review of requests for CIFASD data from external researchers and provide de-identified data to approved researchers.	03/31/2022	80%
c. Maintain an online CIFASD volunteer registry to consent subjects interested in participating in future research studies.	03/31/2022	100%

1) Major Activities and 2) Objectives. We have focused on specific portions of each of the specific aims.

Aim 1: Develop a web portal with a novel, online consenting process to create a large CIFASD cohort for new studies. Online recruitment for this study launched in July of 2018. Since that time, we have recruited and enrolled 117 participants. In addition, we have collected 2D facial images and saliva samples from 41 study participants. Our online informed consent allows us to re-contact study participants for the purposes of recruiting and consenting individuals for other studies, including online intervention protocols.

Aim 2: Perform whole exome sequencing in a targeted set of the newly recruited online CIFASD cohort with features consistent with a high or low probability of Fetal Alcohol Syndrome (FAS) or FASD. As yet, there is an insufficient number of new participants recruited as part of Aim 1 to perform a round of whole exome sequencing. Therefore, the emphasis continues to be on the analysis of whole exome sequencing data that was generated as part of the previous CIFASD collaborative grant (U01AA014809: 3D Facial Imaging in FASD).

Aim 3: Maintain a central informatics resource to manage sharing of data within CIFASD and with the broader research community. We have been working with each project to develop a comprehensive and

complete data dictionary that describes all data fields sent by the project. Once the data files and data dictionaries are reconciled, projects will send data quarterly to Indiana University for central storage. A REDCap database will then be available to CIFASD, and to external researchers who request access to the data.

3) Significant Results.

Aim 1: Approval for the online web portal was received from our Indiana University IRB. Participants can now consent, or in the case of children watch a video and then provide assent, online through the web portal. Participants complete case report forms in REDCap to collect information on demographics, previous medical evaluations for FASD, prenatal alcohol and other drug exposure, and other relevant information. A link is sent to participants to upload 2D facial images to be analyzed by Mike Suttie (U01AA014809: Image Analysis of Neurofacial Effects of Prenatal Alcohol Exposure). We have implemented the use of GUIDs as identifiers in this study to facilitate the transfer of data across multiple projects.

To date, 117 participants have enrolled in the study. We have implemented a number of strategies to increase study enrollment. We have simplified our online consent process and case report forms based on feedback from participants in the FASD community. FASD community organizations such as the National Organization for Fetal Alcohol Syndrome (NOFAS) advertise our study in their monthly newsletter to assist us in recruitment. In addition, NOFAS hosted a live webinar about the study. We have created a recruitment flyer and sent it to FASD clinics throughout the United States and Canada requesting that they distribute the flyer in their clinics and to their patients. Additionally, we have developed electronic study advertisements and have asked online FASD organizations to share these advertisements. We have also created social media accounts on Facebook and Twitter. We recently developed and launched paid advertisements on Facebook in an effort to increase enrollment. We also revised our IRB protocol to enable us to recruit participants from clinics, conferences, or other events. We will attend the 8th International Conference on Fetal Alcohol Spectrum Disorder in Vancouver in March, 2019 and hope to recruit and enroll participants during this conference.

Aim 2: Because of the inherent lag between participant recruitment, saliva collection, DNA extraction and return of results from whole exome sequencing (WES), data from the previous CIFASD collaborative grant (U01AA014809: 3D Facial Imaging in FASD) were returned during the current grant period. These data have now been cleaned and after quality control, we have WES data on 216 unrelated individuals for a range of 7,434 to 145,776 variants, depending on the type of variant annotation used. **All individuals have prenatal alcohol exposure**: 46 with FAS (21.3%), 104 (48.1%) with a deferred status, and 66 (30.6%) with a diagnosis of no FAS. This sample is unique in that there is an even split between 108 European American (50.0%) and 108 African American (50.0%) individuals. A subset of these individuals have brain imaging and MRI data (N=101) and neurocognitive measures (N=197). Almost all individuals have 3D facial imaging data.

Preliminary analyses compared two groups of subjects with prenatal alcohol exposure (FAS vs no FAS). WES is designed primarily to test the effect of rare variants in the coding region of genes; therefore we used the statistical analysis program, RVTEST, to perform a gene based test of the effect of rare variants. We did not limit analyses to a single racial/ethnic group, and therefore included the first four principal components derived from the WES set of common variants (minor allele frequency (MAF) ≥ 0.10) to account for genetic differences in racial background. To maximize the power for our gene-based analysis, we first evaluated genes identified from the animal models led by Johann Eberhart and Scott Parnell. These genes include: *BAX, SESN2, RPTOR, TP53* (BAX and PI3K/AKT/mTOR pathways), *NR5A2, EFCAB7, DYNLT1, RHOB, NNT, KIT, WT1, WDFY1,* and *PTHLH*. Although variants in *PDGFRB* (p=0.041), *NNT* (p=0.0088), and *RPTOR* (p=0.0055) were modestly associated with FAS status, these results did not survive correction for multiple testing.

Secondary analyses examined genome-wide gene-based results to identify the top 3 genes which survived multiple genome-wide testing: *CRIPAK* ($p=4.5x10^{-7}$), *HTT* ($p=6.9x10^{-5}$) and *KIF2A* ($p=1.0x10^{-4}$). All three genes are in the mTOR pathway. We then expanded the comparison to utilize everyone with available WES and examined brain volumes for regions identified in the literature for these 3 genes, as well as seven neurocognitive measures. Preliminary results demonstrate that carriers of a variant in *CRIPAK* have lower hippocampal volume (p=0.0013), and perform worse on a spatial working memory task (p=0.025). Although there were no differences in brain volume measures (caudate or cortex, p>0.60), carriers of a variant in *HTT* performed worse on two different spatial working memory tasks ($0.0052 < p < 0.05$). The rare variants in *KIF2A* demonstrated a protective effect in that carriers had increased caudate (p=0.0012), hippocampus (p=0.013), and cortex (p=0.048) volumes,

but no differences in executive function measures were identified. Further analyses utilizing MRI data as well as 3D image data are currently underway.

Aim 3: All participants in the online CIFASD volunteer registry were informed of our DiG FASD study. We then contacted all of the CIFASD volunteer registry participants who consented to be contacted about our study. Since all the CIFASD volunteer registry participants have been informed of the DiG FASD study and our online web portal is enrolling participants, the CIFASD registry is no longer necessary. We are working to close the CIFASD registry protocol and will leave the website open but provide a link directing individuals to the DiG FASD web portal. All advertisements for the CIFASD volunteer registry have been replaced with advertisements for the current study. In addition, to further our efforts to provide aggregate data and uniform infrastructure across projects we have implemented the use of Global Unique Identifier (GUID) numbers. These universally unique identifiers provide a way to identify the same individual participating in multiple projects. In addition, data can be shared in a de-identified fashion while allowing for the ability of data from the same individual to be studied in aggregate.

4) Key Outcomes and Other Achievements.

Because we examined the entire genome with the WES data, we were able to look up results for genes identified by the mouse and zebrafish genetics components (Parnell and Eberhart). As the immune (Weinberg) and cytokine (Chambers) projects progress, we will be able to compare results with those projects as well. Furthermore, the identification of three genes in the mTOR pathway lend credibility to what has been shown by the mouse and zebrafish models, that this is a key pathway in cell survival and growth factor signaling, known to be disrupted by prenatal alcohol exposure (Eberhart& Parnell, 2016).

Candidate genes will continue to be screened for additional phenotypes, as identified by other CIFASD projects. This project has provided the opportunity for training and professional development for Leah Wetherill, who completed her dissertation in December, 2018. Her dissertation examined the genetic underpinnings of prenatal alcohol exposure. This project has also provided the opportunity for training and professional development for Julian Boes, who is a freshman at Purdue University, majoring in statistics.

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

Professional Development: As a result of her involvement in this project, Dr. Leah Wetherill has completed her PhD in Addiction Neuroscience. Dr. Wetherill and Dr. Elizabeth Rowe attended the Research Society on Alcoholism (RSA) conference in June, 2018 (including the FASD Satellite meeting). In 2019, they will attend the RSA conference in June as well as the 8th International Conference on Fetal Alcohol Spectrum Disorder in Vancouver in March. This will provide an opportunity to increase their knowledge in the field of FASD in general, and more specifically with new research developments, as well as to meet and interact with key FASD researchers in the field. Dr. Tatiana Foroud was part of a submitted symposium at ISBRA which highlighted results from the genetic analysis. This project has also provided the opportunity for training and professional development for Julian Boes, who is a freshman at Purdue University, majoring in statistics.

Dissecting the Genetic Contributions to Fetal Alcohol Spectrum Disorders (DiG FASD)

Interrelation with the aims of the CIFASD consortium and its other projects and update on the progress of those interactions.

One of the aims of the CIFASD consortium is to identify risk and resiliency factors, including genetic influences, on the effects of prenatal alcohol. Identifying these risk/resiliency factors and genetic influences will improve understanding of the mechanisms of alcohol's damaging effects as well as lead to more targeted interventions. The DiG FASD project will provide a genetic basis for investigating these influences in humans and will work with the basic research projects in CIFASD focusing on animal models (Eberhart/Parnell, Hashimoto/Torrii). Further, this project has relationships with CIFASD projects that seek to improve identification of individuals who have been affected by prenatal alcohol exposure (Chambers, Coles, Jones, Mattson, Hammond, Wozniak, Weinberg), neurobehavioral screening (Mattson), and interventions (Petrenko/Tapparello). Interactions with these other CIFASD projects is dependent on building a large participant population. Enrollment in this project has begun and improvements in recruitment and enrollment have been implemented so as to increase participant enrollment.

This project also plays a pivotal role to connect all CIFASD projects. The DiG FASD group works with each project individually to create a viable dataset with an accompanying data dictionary. These files are then implemented into REDcap to facilitate data sharing within CIFASD as well as externally.

Inclusion Enrollment Report

Inclusion Data Record (IDR) #: 174052

Using an Existing Dataset or Resource: Yes

Delayed Onset Study ?: No

Clinical Trial: No

Enrollment Location: Domestic

NIH Defined Phase III Clinical Trial: No

Study Title: Dissecting the genetic contributions to fetal alcohol spectrum disorders

Planned Enrollment

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/Alaska Native	25	25		12	12					**74**
Asian	10	10		2	2					**24**
Native Hawaiian or Other Pacific Islander	17	17		3	3					**40**
Black or African American	120	120		21	21					**282**
White	668	668		122	122					**1580**
More than One Race	0	0		0	0					**0**
Unknown or Not Reported										
Total	**840**	**840**		**160**	**160**					**2000**

Cumulative Enrollment

This study involves an existing dataset and does not include cumulative enrollment.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	Dou X, Menkari C, Mitsuyama R, Foroud T, Wetherill L, Hammond P, Suttie M, Chen X, Chen SY, Charness ME. L1 coupling to ankyrin and the spectrin-actin cytoskeleton modulates ethanol inhibition of L1 adhesion and ethanol teratogenesis. FASEB journal : official publication of the Federation of American Societies for Experimental Biology. 2018 March;32(3):1364-1374. PubMed PMID: 29109170; PubMed Central PMCID: PMC5892731.
Complete	Suttie M, Wozniak JR, Parnell SE, Wetherill L, Mattson SN, Sowell ER, Kan E, Riley EP, Jones KL, Coles C, Foroud T, Hammond P. Combined Face-Brain Morphology and Associated Neurocognitive Correlates in Fetal Alcohol Spectrum Disorders. Alcoholism, clinical and experimental research. 2018 September;42(9):1769-1782. PubMed PMID: 29935097; PubMed Central PMCID: PMC6120799.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Category	Explanation
Research Material, Audio or video, Evaluation Instruments, Educational aids or curricula, Survey Instruments	Website: https://digfasd.org The https://digfasd.org web portal was created as part of Specific Aim 1 of this project. It serves to recruit and enroll participants as well as disseminate information about the project.

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? No

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization?

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

Foroud U01 Genetics - Monthly Ns	Current Month	Cumulative Total	May 2019 Goal	% to Goal	Overall Goal	Completion Goal Date	May 2018 Goal	May 2019 Goal	May 2020 Goal	May 2021 Goal	May 2022 Goal
Online Web Portal Participants - Date Web Portal 'opened' = 7/5/2018											
Number of participants enrolled and consented in the Web Portal	13	117	600	19.5%	2000	7/31/2022	0	600	1200	1800	2000
Participants who uploaded 2D facial images	10	52	500	10.4%	2000	7/31/2022	0	500	1000	1500	2000
Participants who returned saliva samples	8	41	500	8.2%	2000	7/31/2022	0	500	1000	1500	2000
https://digfasd.org/							Cumulative - at the end of each year.				

Foroud U01 Genetics - Task Percentages	% Complete	Completion Goal Date
DiG FASD Aim 1: Develop web portal YEARS 1 and 2		
Develop consent form	100%	1/31/2018
Develop Case Report Form (study questionnaire)	100%	1/31/2018
Develop procedure for collecting prior FASD evaluations for participants	100%	2/28/2018
Develop saliva collection system	100%	3/31/2018
Develop assent video	100%	4/30/2018
Finalize data dictionary with CIFASD Informatics Core	100%	4/30/2018
Establish file transfer with CIFASD Central Repository	100%	9/30/2018
Develop protocol, video and interface for collecting 2D facial images	100%	4/30/2018
Develop risk score from facial imaging data	0%	10/30/2018
Develop study web portal	100%	4/30/2018
Final IRB approval for protocols, consents/assents, HIPAA and materials	100%	5/31/2018
Publicize study through NOFAS, support groups, FAS-related websites	100%	8/30/2018
Implement FONS (in collaboration with S. Mattson)	0%	3/31/2019
DiG FASD Aim 2: Whole Exome Sequencing (WES)		
Select samples for WES	0%	2/15/2019
Saliva samples sequenced at IU Sequencing Core Cumulative Goal Ns YR2 =100, YR3 = 250, YR4 = 600, YR5 =700	0%	3/31/2019
Genetic analyses performed in CIFASD4 project nominated genes	0%	5/1/2019
Genome-wide genetic tests performed in CIFASD4	0%	5/1/2019
Genes of interest shared with other CIFASD4 projects	0%	5/31/2019

	Start	End
Current month (defined by project) =	1/15/2019	2/22/2019
Date of project numbers update entry =	2/22/2019	

DiG FASD Project Update

Web-based Participation	*n*
Participants Consented	92
Participants with 2D Facial Images	36
Participants with Saliva Samples	28

Project Improvements to Increase Accrual:

- Revising IC to recruit in clinics (includes C. Petrenko)*
- Shortened consent form based on participant feedback*
- Updated web portal*
- Facebook ads to recruit participants*
- Developing consent video for adults

** Submitted to IRB*

CIFASD Central Repository

- CIFASD3
  - Data is stored in REDCap
  - Ready for distribution to approved researchers outside CIFASD
- CIFASD4
  - Everyone has provided a data dictionary
  - Everyone has uploaded some type of file to their IU Box Project Folder
  - Actual data file(s) should be uploaded by December 15, if applicable
  - CR team will reconcile data dictionary with data file for each project
  - Project-specific feedback will be provided

CIFASD4 Face-to-Face Mtg. Progress Report MAR2019

Principal Investigator(s): Michael Suttie and Alison Noble
Institution(s): University of Oxford
Title: Image Analysis of Neurofacial Effects of Prenatal Alcohol Exposure
Grant Number: 5 U01AA014809-15

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

1. Automated screening of facial images for effects of prenatal alcohol exposure with potential for on-line and mobile device use and integration of genetic, behavioural and cognitive data.

2. Improved analysis of face-neurocognitive-alcohol interactions.

3. Fetal ultrasound analysis to detect facial, cranial and neural effects of prenatal alcohol exposure with neonatal follow-up.

There have been no major changes in these goals.

B.2 ACHIEVEMENTS AGAINST GOALS

Automated screening of facial images for effects of prenatal alcohol exposure with potential for on-line and mobile device use and integration of genetic, behavioral and cognitive data

In CIFASD4, a primary objective is to assist clinical diagnosis of FASDs by automating facial analysis and providing flexibility in terms of when, where, by whom and with what user effort the image capture and analysis is carried out and reported. This will mean being able to handle 2D and 3D photographs, different modes of capture (webcam, conventional camera, smartphone and tablet), and multiple "diagnostic scenarios"; for example:

1) CIFASD online recruitment
    a. lay access to web portal
    b. 2D image
    c. no immediate user feedback
    d. later offline analysis by researcher

2) FASD clinic
    a. smartphone
    b. 3D image
    c. semi-automated analysis on laptop
    d. immediate diagnostic feedback to clinician.

<u>3D FACIAL ANALYSIS</u>

We have worked closely with Dr Raja Mukherjee who runs the only FASD clinic in England (there is one other FASD clinic in the UK, in Scotland). With the co-operation of referred families and Dr Mukherjee's clinical colleagues, observation of clinical sessions has been crucial to deciding how best to integrate our face screening software into clinical workflow. As part of the patient session, the clinician takes a 3D

image is using a high-end handheld system. Our clinical software 'FaceScreen' generates a printable clinical report in pdf format using a semi-automated process. A preliminary analysis of a comparison with the 2D screening tool (Washington) has shown the agreement between the methods, with the additional benefit of 3D being able to identify the presence of secondary features such as malar flattening and retrognathia. To date, the tool provides capability to:

a) compare palpebral fissure length graphically to norms obtained from public sources

b) display heat maps of surface curvature of the face to visualize philtrum smoothness and to some extent malar flattening

c) display heat maps showing normalized difference to age-matched controls to quantify minor anomalies such as retrognathia and malar flattening.

d) apply the above methods to a range of ethnicities (Caucasian, Cape Coloured & African American (in progress)

An initial testing phase of our clinical software at Dr Mukerjee's clinic has uncovered some technical issues, as the software is based on a platform initially developed over a decade ago. As a result, the tool experiences certain usability, performance and latency issues on newer versions of Windows. Development this year has focused on rebuilding the software interface on an updated platform, with enhanced integration for 3D camera systems (to include Canfield H1, Bellus tablet based systems and iPhone X) and multi-processor utilization. Implementation of the new interface is underway (fig 1), but will require further development and testing in Y3. If successful, funding from an administrative supplement application will accelerate this process with the recruitment of an additional technical role at the University of Oxford.

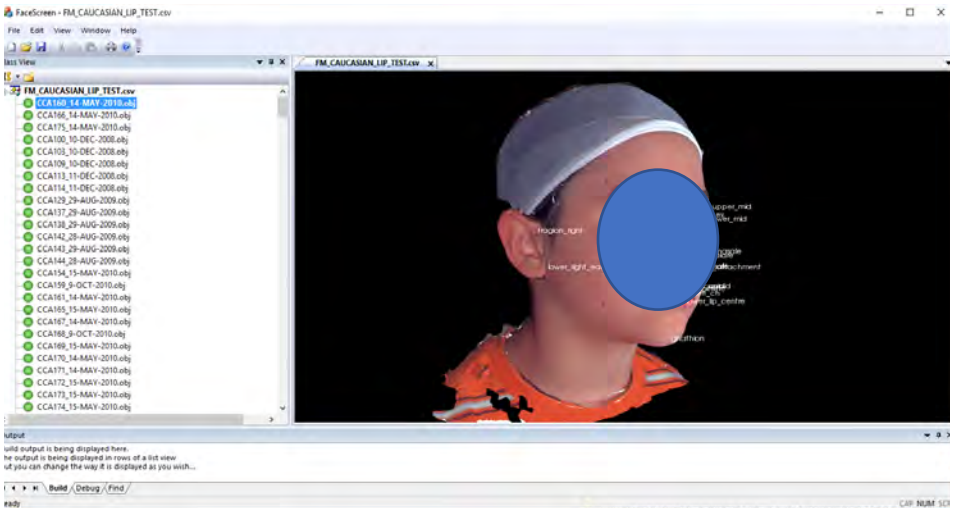


Fig 1 New clinical screening software interface

African American 3D Face Analysis

For the screening tool to cover a range of ethnicities, we need to ensure that those populations are covered by prior analysis. This is to provide meaningful classification training sets to support control-FASD discrimination testing, and provide appropriate ethnically matched normative control data. Previous published CIFASD studies focused on Caucasian and Cape Coloured populations, but the African American analysis had been missed due to a lack of data. However, there now sufficient numbers to

recapitulate previous studies in the latest phase of CIFASD (controls n=91; deferred n=134; FAS n=20; exposed (no FAS) n=20; age range 5-18yrs). Preliminary results from a surface based analysis suggest the facial features of individuals with FAS are identifiable using 3D analysis, with control-FAS discrimination rates being mostly concordant with our previous Caucasian and Cape Coloured studies. As expected, mean differences in the FAS group show a significant growth reduction (fig 2.a), which dilutes other notable features. To overcome this we scale the FAS mean to identify more subtle dysmorphism. When looking at surface-base differences of the scaled-FAS mean on the z-axis (depth) we observe significant retrognathia (fig 2. b). On the Y-axis (vertical), the thin upper lip becomes visually apparent. When looking at the deferred group mean, there is a significant reduction in facial growth, however, specific and homogenous features are difficult to distinguish. To overcome this, we use facial signature graph analysis, which allows the individual assessment and quantification of facial dysmorphism across the set. We built a graph containing individuals with a FAS (n=17), or a deferred (n=82) clinical label within a suitable age range for the control set (fig 3). Much like previous analysis performed on heavily exposed individuals without criteria for a FAS diagnosis, the deferred group splits into two subgroups. Dividing those labelled as deferred into a group with close affinity to the FAS individuals (n=48) and another, more control like group (n=34). Interestingly, like the Cape Coloured analysis we observe neurocognitive measures in the FAS-like group that are significantly lower than the more control like group (DASII Verbal $p<0.03$; DASII GCA $p<0.03$). This result needs to be confirmed with randomly sampled sets as the numbers with neurocognitive test scores are low (n=25 FAS like group; n=18 control like group).

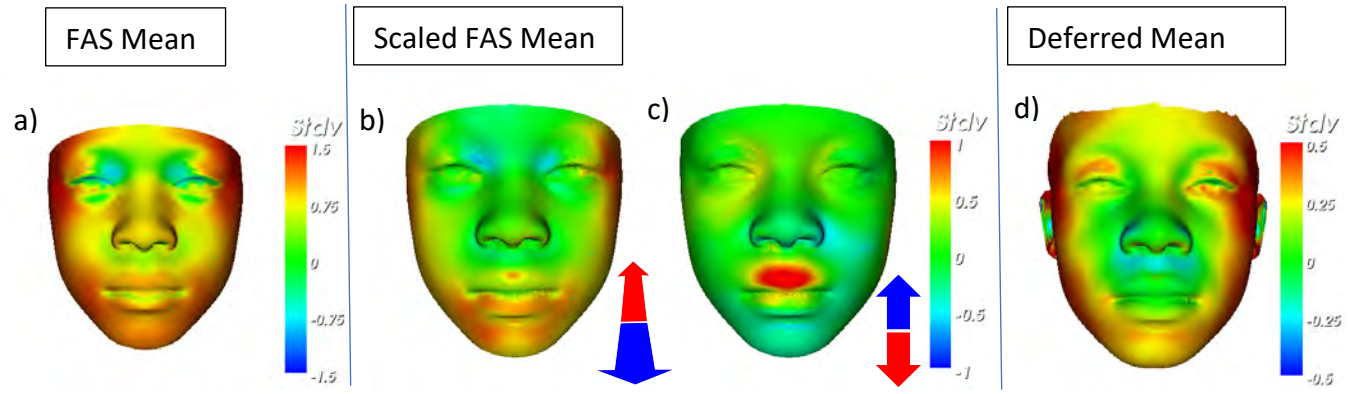


Figure 2 a) African American FAS mean – facial features dominated by red (deflation compared to control) indicating severe growth reduction. b) Scaled FAS mean on relative to a z (depth) axis showing retrognathia with red area on the chin c) Scaled FAS-mean on Y (vertical) axis showing lip vermillion thinness. d) Deferred mean, overall significant reduction in size.

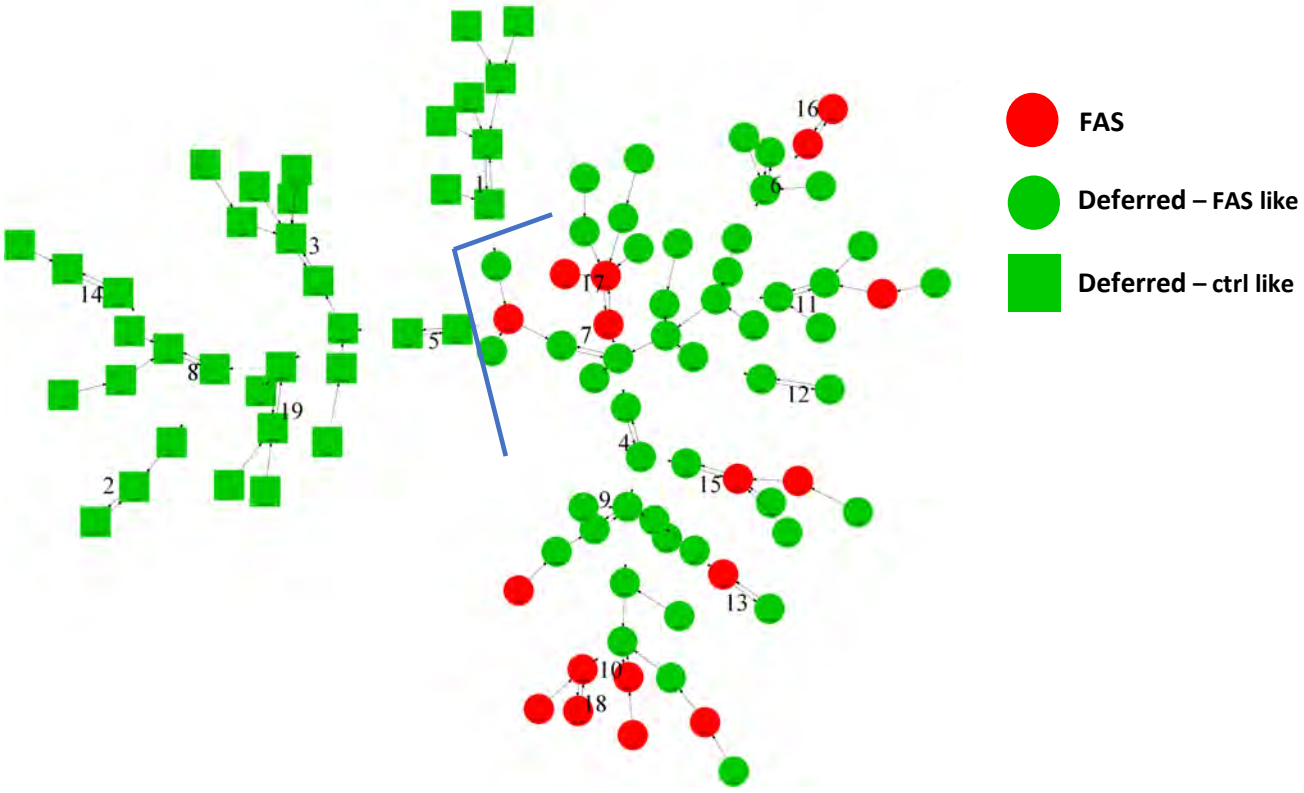


Figure 3 Facial signature graph colour coded as FAS (red, n=22) and deferred (green, n=82) with individuals normalized against 35 age-match control means. The deferred group divide into two distinctive sets, those with an affinity to the FAS individuals (green circles) and those who move away from the FAS. The graph is splits left from right by two secondary links.

AUTOMATED 3D LANDMARKING

For the automation of facial analysis, we first have to overcome a major technical hurdle being the automated placement of anatomical landmarks. This is a requirement to derive accurate measurements, perform surfaced-based shape analysis, and provide representations of directional curvature for the analysis of the philtrum. With the assistance of Canfield Scientific Inc, the hand-held 3D camera manufacturer, we have been able to utilize embedded functions for automated landmark placement. Testing on existing images, we have shown the ability to produce these points, however, this method is restricted to images captured using only the Vectra H1 from Canfield.

To date, there are a number of published shape and curvature based techniques for the automated identification of anatomical landmarks. We have tested a multitude of these using 3D image data from earlier CIFASD phases. Unfortunately, all available techniques require some form of manual pre-pre-processing, involving image cropping and re-orientation. As an alternative, we have focused on utilizing more sophisticated machine learning tools to overcome this issue. Dr Roubing Huang has been developing and testing methods based on Convolutional Neural Networks (CNNs) for the automated annotation of 2D facial landmarks. By utilizing manually placed landmark data from previous 3D CIFASD studies, we have been building training sets and performing the automated placement on the 2D texture images which accompany the 3D image data. Recently, we have developed a method for transferring points from the 2D images to their corresponding location on the 3D surface (fig 4).

We have implemented a method to utilize the 2D capture from 3D camera systems to develop and train deep learning algorithms. Preliminary results of this methodology are positive, but more testing is required. Technical aspects of this work is to be submitted as an abstract to the international Conference on Medical Image Computing and Computer Assisted Intervention later this year and additional analysis/progress using this will be presented as the RSA in June.

Figure 4 Automated landmark detection using 2D image textures from the 3D acquisition. Left: 2D texture image acquired from a stereo-photogrammetric 3D camera. Initial facial recognition algorithms (green box) finds the two sides of the face, detects orientation and crops, before processing using pre-trained CCNs to place anatomical landmarks based on that of the training set. Texture coordinates map to the 3D surface, and using this mapping information we transfer the located landmarks to the 3D surface.

2D FACIAL ANALYSIS

In CIFASD4, 2D facial photographs will be collected through the web-based recruitment portal being produced at Indiana University. Other CIFASD4 collaborators have also been collecting 2D images using conventional cameras. CIFASD colleagues have followed the 2D imaging protocol proposed in Y1, and recruitment intake has been increasing steadily over the year. In Oxford, we have established a secure upload portal (FILR) for the transference of all image data to us - currently utilized by all members capturing 2D and 3D images. To date, the quantity of images is not sufficient for analysis using deep learning techniques proposed by Chris Nellåker here at Oxford, however, plans are in place for analysis in the coming year.

2D profiles as a pseudo 3D profile model to build a shape model

We have created and image processing pipeline (fig 5) for the semi-automated extraction of profiles from 2D images. The extracted 2D profile curves can be converted to ribbon-like 3D surfaces as previously used in 3D profile shape models within CIFASD3. Multi-folded control-FAS discrimination testing for this derived face profile with 6 landmarks produced concordance with clinical diagnosis of over 95%. So far, utilizing CIFASD4 2D images provided by consortium members we have successfully produced a test model (n=30). As image numbers increase, they will added to this model until there is a sufficient quantity to perform analysis.

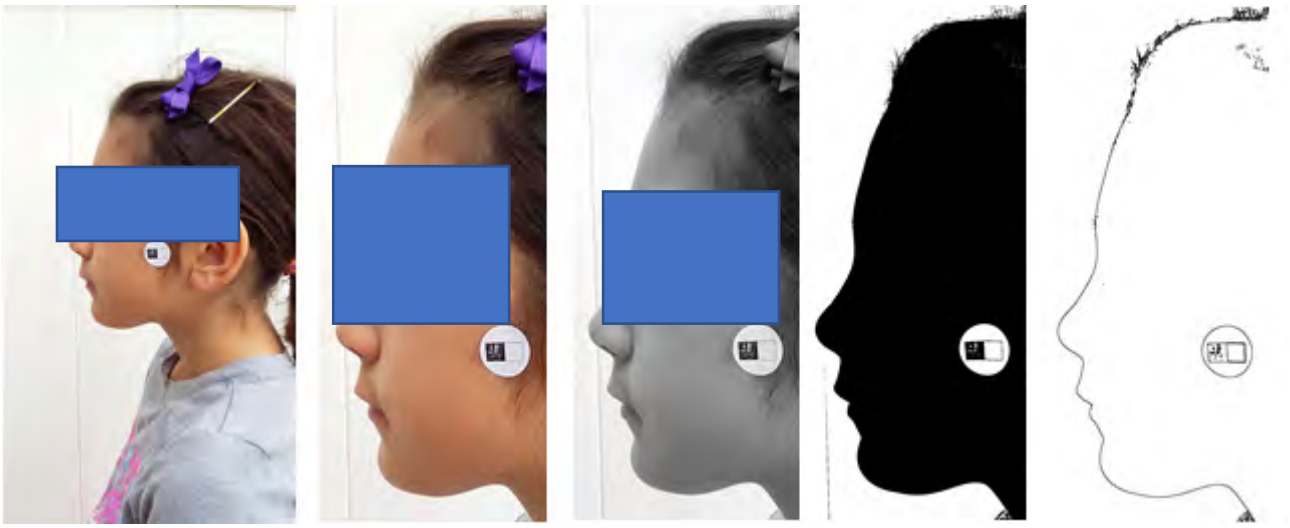


Figure 5 2D profile image semi-automated processing pipeline. Left to right: Initial image is cropped and scaled using the stickers as a reference for dimensionality and converted to 8-bit greyscale image. A mask is generated and holes are filled suing dilute and erode binary functions (second from right) before an edge detection algorithm leaves only the outline of the profile (far right). This profile outline is a series of 2D points, which convert to a ribbon like 3D mesh for analysis.

Fetal ultrasound analysis to detect facial, cranial and neural effects of prenatal alcohol exposure with neonatal follow-up

FETAL BRAIN STRUCTURE IDENTIFICATION IN OXFORD

Dr Ruobing Huang started work on the project supervised by Professor Alison Noble at the end of Y1. Her thesis focused on standard foetal neurosonography examinations for evaluating key brain structures from their identification and segmentation within ultrasonographs.

Localizing neural structures in 3D US is non-trivial as image quality is greatly affected by the presence of speckle; foetal brain structures change in size and shape continuously over gestation age; and foetuses can be randomly positioned in the uterus with the skull having variable orientation. In her doctoral project, Ruobing Huang has been developing automatic methods using state of the art machine learning techniques, such as random forest classifiers, to localize the corpus callosum and other structures of relevance to the CIFASD consortium.

In addition to this, we are aiming to conduct shape analysis of the corpus callosum using dense surface modelling, in a similar manner to the techniques used in our face-brain study. This analysis uses more a traditional approach, manually outlining the corpus callosum from a mid-sagittal slice from a transfontanelle ultrasound. Dr Huang has developed a tool for the manual segmentation of the corpus callosum from volumetric images (fig 6), which appropriately formats the data points ready for analysis. The manually traced segmentation converts into a ribbon like 3D mesh ready for processing and building into a surface-shape model. Given the availability of processed corpus callosum data from our face-brain study, we will be able to compare shape differences of the corpus callosum to those identified previously in the adolescent population.

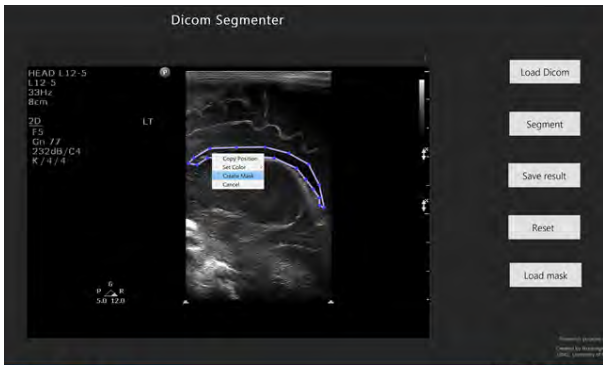


Figure 6 Software tool created for the manual segmentation of corpus callosum in transfontanelle ultrasound images

NEONATAL FACE ANALYSIS IN BRIGHTON

The subcontracted collaboration with Dr Neil Aiton on neonatal facial analysis has made significant progress over the past 10 months. Carryover/surplus funds made available have allowed for the recruitment of a second imaging assistant to capture 3D facial images at an additional site. This has essentially doubled recruitment capacity.

At present, we have 3D images for 240 neonates with accompanying alcohol exposure information collected retrospectively. Preliminary analysis on around half (n=134) of this dataset has shown the ability of our software to recognise some of the subtle features previously described in our facial studies. Current analysis is performed on a case-by-case basis focusing on those with known alcohol exposure. As a result, we have presented a dysmorphology analysis to Dr Aiton to confirm the presence of features in a small subset. These individuals are deemed to be at high risk of FASDs and will be monitored closely. The facial signature of one of these cases (fig 7), shows significant facial differences, including; retrognathia, mid-facial hypoplasia, nasal flattening and lateral size differences. There is a close resemblance to the facial signatures of FAS/FASD, and in a signature graph analysis, this individual has an affinity to FAS-dominant clusters. We aim to publish findings once recruitment has finalized.

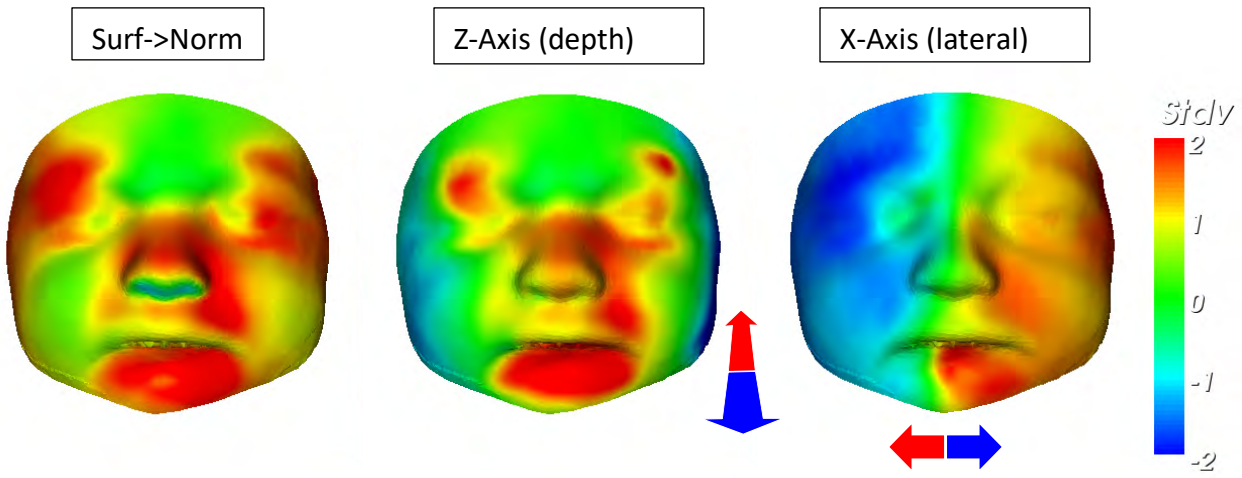


Figure 7 Facial signature heat maps for an individual neonate with confirmed alcohol exposure. Left: Surface to normal differences showing significant reduction (red) in the temporal, malar (midface), nasal and gnathion (chin) regions. Middle: Same differences shown on the depth axis. Right: Significant lateral reduction in face with asymmetry on the chin.

B.3 Competitive Revisions/Administrative Supplements

An application for an administrative supplement is in the process of submission after initial approval to proceed. This is to propose an acceleration of clinical translation of our face analysis techniques in line with the U01 goals.

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

Michael Suttie was awarded the position of co-PI, providing an elevated position within the project and significant career progression.

Indirectly, the project is providing training opportunities for clinical partners and collaborators in using the Canfield hand-held 3D camera and face screening tools (Dr Raja Mukherjee; Dr Neil Aiton).

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

In addition to the usual academic publishing route and presentations at specialist conference meetings organized under the FASD UK, EUROFASD, Canadian FASD and RSA, other dissemination opportunities have arisen:

FASD Research Network, Planning Meeting, Salford (UK)

Dr Raja Mukherjee, Dr Neil Aiton, and several other FASD researchers have planned to setup a research network to better connect FASD researchers in the UK. Michael Suttie was invited to present material on FASD face-brain analysis and provide an insight into the work of CIFASD at this meeting.

NEURODEVELOPMENTAL Disorders: Causes, Identification and Outcomes

Michael Suttie was invited to attend this meeting focusing on neurodevelopmental disorders, and present work on 3D face and brain analysis for FASD and other genetic conditions.

Biomedical Imaging Cluster Series, Oxford

Ruobing Huang presented a talk titled: Understanding Neurofacial Effects of Prenatal Alcohol Exposure: an image analysis approach in a seminar series at the University of Oxford.

B.6 What do you plan to do during the next reporting period to accomplish the goals?

For the four-month period February to May 2019, we plan to do the following:

- continue to test face screening tool in the FASD clinic, set up a validation study
- continue to improve automated landmarking methods using machine learning tools, and test on previously analyzed CIFASD datasets. Recapitulate earlier studies to determine efficacy
- continue the African American face analysis and integrate this population into the clinical software with a view to publish findings
- process neonatal 3D face images to add to current analysis with a view to publish findings
- advertise a job role for an additional technical assistant for the acceleration of the clinical software
- provide Oxford colleague Chris Nellåker with the collected 2D images so that he can test his customized 2D facial analysis software for control-FASD discrimination testing

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

Suttie M, Wozniak JR, Wetherill L, Mattson S, Sowell ER, Kan E, Riley EP, Jones KL, Coles C, Foroud T, Hammond P and the CIFASD. Combined Face-Brain Morphology and Associated Neurocognitive Correlates In Fetal Alcohol Spectrum Disorders. Alcohol Clin Exp Res. 2018 Sep;42(9):1769-1782. doi: 10.1111/acer.13820 PMCID: PMC6120799

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

*** Poster Abstracts and Presentations**

1 EUFASD 2018, Berlin – Presentation
2 EUFASD 2018, Berlin – Poster presentation
3 RSA 2018, San Diego – Presentation
4 MICCAI 2018, Granada, Spain – Poster presentation
5 Biomedical Imaging Cluster Series, Oxford – Presentation
6 FASD Research Network, Salford (UK) – Presentation
7 NEURODEVELOPMENTAL Disorders: Causes, Identification and Outcomes - Presentation

Describe your project's interrelation with aims of the CIFASD consortium its other projects and the progress on the interactions.

Tatiana Foroud (Indiana), Clare Coles (Emory) and Joanne Weinberg (Calgary) – Working with these members to collect 2D and 3D image data.

Tina Chambers (San Diego) - Ukraine 3D facial images revisited with a preliminary analysis complete

Sarah Mattson (San Diego) – Planned interaction to discuss the potential of synergy between decision tree and 3D facial analysis tool

Tatiana Foroud, Leah Wetherill (Indiana) – We have recently been working to investigate if any facial differences are apparent from the different gene carrier groups identified from whole-exome sequencing (*KIF2A, HTT and CRIPAK).*

Noble Suttie U01 2D/3D Images	Current Month	Cumulative Total	May 2019 Goal	% to Goal	Overall Goal	Completion Goal Date	May 2018 Goal	May 2019 Goal	May 2020 Goal	May 2021 Goal	May 2022 Goal
2D Images Received											
Web Portal - Foroud	22	55	600	9%	2000	5/31/2022	0	600	1200	1800	2000
Atlanta - Coles	0	0		#DIV/0!							
Ukraine - Chambers	0	0	22	0%	65	5/31/2021	0	22	44	65	65
Vancouver - Weinberg	4	14	100	14%	120	5/31/2020	50	100	120	120	120
3D Images Received											
Minnesota - Wozniak	0	52	60	87%	90	5/31/2020	30	60	90	90	90
Ultrasound and 3D Images Received											
Aiton sample - 135 per year	103	240	270	89%	675	5/31/2022	135	270	405	540	675
Ukraine - Chambers	0		22	0%	65	5/31/2020	0	22	44	65	65

	Start	End
Current month (defined by project) =	1/22/2019	2/25/2019
Date of project numbers update entry =	2/25/2019	

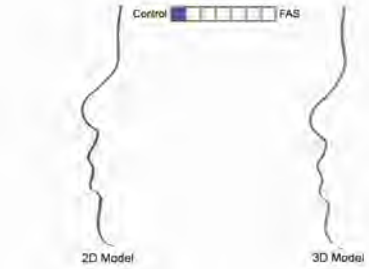
Cumulative - at the end of each year.

Ultrasound Image Analysis (Dr Neil Aiton)

- Data collection: 9 alc-exposed, 7 abnormal, 8 controls – recruitment ongoing.
- Analysis: Software developed by Dr Huang for Corpus Callosum extraction. Protocols for DSM analysis established based on Face-Brain paper (Suttie et al. 2018)
- Developing automatic segmentation algorithm (in progress)
- Awaiting more images for shape analysis

2D Image Analysis

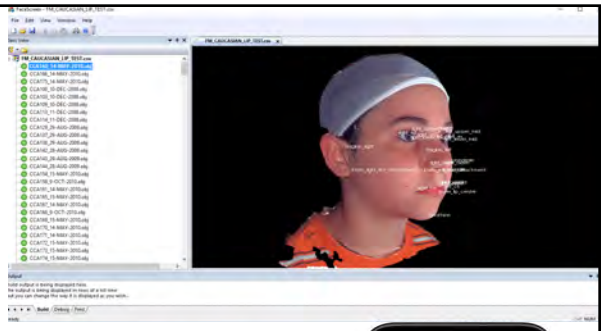
- Implement 2D landmark detection algorithms utilising texture images from 3D acquisition.
- Train machine learning classifiers to predict prenatal alcohol exposure (in progress)
- Analysis protocol for profile extraction from 2D images successful.
- 2D images from CIFASD members being uploaded monthly



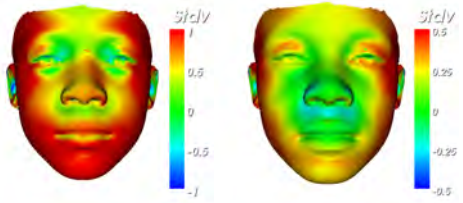
[1] Viola, P. and Jones, M., 2001. Rapid object detection using a boosted cascade of simple features. In *Computer Vision and Pattern Recognition, 2001. CVPR 2001. Proceedings of the 2001 IEEE Computer Society Conference on*(Vol. 1, pp. I-I). IEEE.
[2] Newell, A., Yang, K. and Deng, J., 2016, October. Stacked hourglass networks for human pose estimation. In *European Conference on Computer Vision* (pp. 483-499). Springer, Cham.

3D Face Analysis

- Neonatal subject recruitment capacity **doubled** in Brighton
- New version of screening software in development
  - GUI/usability improvements
  - Multi-core utilisation
  - Integration with camera software
  - Integration with Canfield automated landmarking



- African American analysis (In progress)



FAS Deferred – alcohol exposed

Lip-philtrum Models

- Multi-SVM classification for lipometer scores.
  - 1-2 82%
  - 3 79%
  - 4-5 83%

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The overarching goal of the current proposal is to improve identification of individuals affected by prenatal alcohol exposure. As part of the current funding period, the PI and collaborators developed an evidence-based Decision Tree to more effectively identify alcohol-affected children. The work was conducted in a research setting in a sample at high risk for having an FASD. The proposed project extends this work by using novel electronic data collection techniques and subjects recruited from outpatient clinical settings, from population-based and international cohorts, and through a web-based recruitment portal. The proposed research meets the RFA objective of improving clinical case recognition with the following specific aims:
1.Explore the clinical utility of the CIFASD Decision Tree using multiple methods and samples
a.Explore utility of the CIFASD Decision Tree using data from lower risk samples. Existing data will be used to further validate the Decision Tree in two heterogeneous samples. Analysis of these data early in the project will guide revisions to the Decision Tree if needed. Two data sets will be examined:
i.CoFASP (Chambers PI). These data were collected as part of a prevalence estimate in first grade students in San Diego, California. Analyses will test the validity of the Decision Tree in a lower-risk population-based cohort.
ii.CIFASD Ukraine (Chambers U01). The Decision Tree will be applied to data collected in the Ukraine cohort during CIFASD 3 Subjects were age 4 at the time of assessment and analyses will test the downward extension of the Decision Tree in an international sample.
b.Explore feasibility, sensitivity, and specificity of CIFASD Decision Tree in clinical settings.
i.Ensure consistent evaluation of subjects at all CIFASD Sites. This shared aim (with Dysmorphology Resource lead by Dr. Jones) will ensure that subjects recruited at multiple sites will be examined with standardized physical and neurobehavioral measures, including the Decision Tree. Physical and neurobehavioral data will be collected at two sites (California/Minnesota). This aim relates to Specific Aim 1 of Dr. Jones' Dysmorphology Resource.
ii.Develop an internet-based or mobile app version of the CIFASD Decision Tree for identification of children affected by prenatal alcohol exposure. An electronic version of the Decision Tree (eTree) will be developed and deployed in clinical settings. Programming support will be provided by the Administrative Resource and Dysmorphology support will be provided by the Dysmorphology Resource. Electronic versions of required measures will be built into the eTree and administered on an iPad; results will be available to the clinician. Previous use of the Decision Tree took place exclusively in research settings and in a retrospective fashion (i.e., with subjects known to be alcohol-affected). FASD clinics will serve as initial test sites. Additional testing will be conducted in general outpatient psychiatry and behavioral pediatric settings. Clinical validity, sensitivity, specificity and barriers to implementation will be assessed.
c.Validate results of the CIFASD Decision Tree using advanced neuropsychological data. Laboratory-collected neuropsychological data will be collected on a subset of subjects assessed with the Decision Tree to validate the eTree classification in these clinical settings. Specificity will be tested by including subjects without histories of prenatal alcohol exposure with and without behavioral concerns or conditions (see aim 2b).
2. Develop, implement, and validate online neurobehavioral screening tools for use with subjects recruited through the CIFASD web portal.
a.Develop and implement a novel online neurobehavioral screening tool. Based on our extensive experience with the neurobehavioral profile of FASD, we will develop the FASD Online Neurobehavioral Screen (FONS) that can be self-administered in an unsupervised setting. This tool will be implemented as part of the web portal developed as part of Dr. Foroud's U01 project. In conjunction with Dr. Foroud and Dr. Hammond, neurobehavioral data will be combined with facial image data to develop a risk score to quantify the likelihood of FAS or an FASD. Risk level can be used to prioritize subjects for participation in other CIFASD studies.
b.Validate online neurobehavioral tool (FONS) in a subset of subjects. Neurobehavioral data will be collected on subjects recruited at SDSU to validate the results from the FONS, using laboratory-based measures. As in CIFASD 3, three groups will be included, those with prenatal alcohol exposure, non-exposed subjects with other behavioral concerns or conditions, and non-exposed typically developing controls. These subjects will also be screened with the decision tree (see Aim 1c). Additionally, data will be collected using 3D facial imaging to provide validation data to Dr. Hammond's U01 project.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Mattson_CIFASD4_RPRR 2019.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

During the next reporting period, we plan to continue our efforts in meeting all our aims.

Aim 1a: we are examining why the ARND agreement is so low and hope to find a solution that optimizes our ability to accurately identify alcohol-affected youth. We hope to incorporate novel analytic techniques (e.g., Machine Learning, Decision tree, Neural Nets, Random Forest) to accelerate dissemination of the eTree to clinical settings. We are submitting an administrative supplement for this work.

Aim 1b/c: we will continue to collect data in the existing 3 sites and add 2 additional sites.

Aim 2a: this is where we will expend most of our effort. We will develop, pilot, and implement the FONS as part of the WebPortal. As indicated, we plan to enhance the dysmorphology portion of the eTree app to allow direct communication between the app and the central repository and (hopefully) to incorporate automation of percentile conversions.

Principal Investigator: Sarah Mattson
Institution: San Diego State University
CIFASD4 Project Title: Multisite Neurobehavioral Assessment of FASD
Grant Number: U01AA014834

B.2. Major activities

A. **Archival Data Analysis (Aim 1a).** *Analyze existing data from lower risk samples.*
We requested and obtained data from the San Diego coFASP site (Chambers, PI). Data for 897 subjects were entered these data into the eTree app. We previously reported that these results were initially disappointing – approximately 72% of subject were "correctly" classified, i.e., those with a diagnosis on the FASD spectrum were classified as Probable AE by the decision tree. We revised the tree and increased our sensitivity. More recently, however, we used the eTree application to re-analyze the coFASP data. With this approach, our accuracy rates improved to 84% overall. Data are summarized in **Table 1**. We recently found an error in the eTree app that applied only to data uploaded (like the coFASP data) which accounted for the change in results. Even so, as illustrated, the agreement between coFASP diagnosis and the eTree for subjects with ARND needs further investigation. In particular, while the overall agreement is very good (83%), it is not clear why the agreement for subjects with a coFASP ARND diagnosis is so low (28%). By definition, all the subjects with a coFASP diagnosis of ARND have some history of prenatal alcohol exposure. In the coming year, we plan to examine further the discrepancies in these two approaches, particularly as it pertains to ARND. We will also examine the data from the CIFASD Ukraine data.

Table 1. Agreement between CoFASP Diagnosis and eTree Application (Aim 1a).

	CoFASP Diagnostic Group					
Tree Result	FAS	PFAS	ARND	No FASD	Unable to Dx	Total
Proposed AE	**5**	**31**	**13**	101	3	153
Proposed Non-AE	0	12	34	**693**	4	743
Total	**5**	**43**	**47**	**794**	7	896
Agreement	100%	72%	28%	87%	--	**83%**

B. **eTree (Aim 1b).** *Develop and refine eTree application, collect data using eTree application in 3 settings*

C. **Validation Test Battery (Aims 1c/2b).** *Conduct validation neuropsychological testing on a subset of subjects*
We have been successful in recruiting and enrolling subjects in the eTree study for Aims 1b and 1c). We have collected data in 2 cities with a total of 3 sites. In San Diego, subjects are recruited from the UCSD FASD clinic and the SDSU Center for Behavioral Teratology. We added the CBT site to capture a broader range of subject. In Minneapolis, subjects are being recruited/tested under the direction of Dr. Jeff Wozniak, PI of the CIFASD neuroimaging project. Subjects recruited for the neuroimaging project are administered the same validation battery and also assessed using the eTree when a dysmorphology exam is completed. Table 2 summarizes the number of subjects included in this portion of the project.

Table 2. Number of Subjects Recruited and Enrolled in Tree Study (Aims 1b/1c)

Target	San Diego-UCSD FASD	San Diego-CBT	Minneapolis	Total
# Consented/Record Created	72	79	56	207
# Complete in eTree	66	71	26	163
# Partial Record in eTree	6	8	9	23
# Complete NP Validation	85		56	141

While analysis of the neuropsychological validation test battery is premature, we have completed some preliminary analyses examining the relation between the eTree result, Risk Score, and the ARND Checklist developed by Larry Burd. The ARND checklist is a 35-item and results in a yes/no score indicating the presence of ARND. We examined the relation between the ARND checklist and 2 eTree indicators in 101 subjects: eTree Result (yes/no) and Risk Score (0-5). These exploratory analyses are illustrated in the accompanying figures.

Figure 1. Results of ARND checklist by eTree results. More youth are identified as affected using the eTree than the ARND checklist. Indicating a possible increase in sensitivity. Further analysis is needed to test for both sensitivity and specificity.

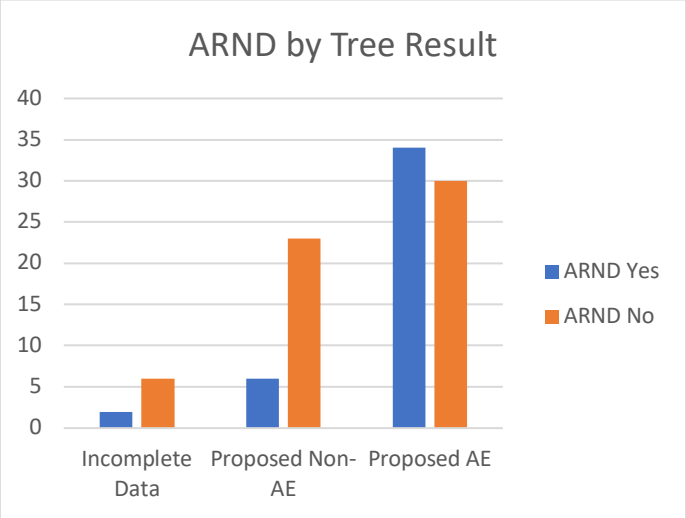
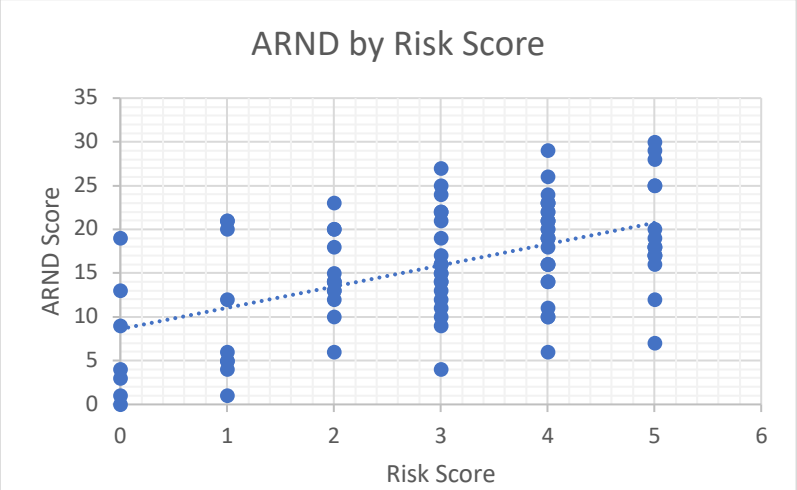


Figure 2. Results of ARND checklist by Risk Score. As ARND score increase, so does risk score (r = .53). Correlations are also significant when history of prenatal alcohol exposure is controlled.



D. **FONS/Web Portal (Aim 2a).** *In Progress: development of FONS*

A significant aim of our proposal is to develop and implement a novel online neurobehavioral screening tool to be integrated into the WebPortal as proposed by Dr. Foroud. Our biggest obstacle this year was with unexpected staffing changes that have dramatically affected this aim (two staff members left to take other jobs to further their academic aspirations and one graduate student had an unexpected and very serious medical event that required surgery). We are behind schedule but are catching up. Based on my review of the literature, initial analytic strategies have focused on behavioral domains/questions that differentiate alcohol exposed subjects from both typical controls (our T-CON group) and clinical controls (our B-CON group). These analyses have identified 5 specific items that can distinguish the groups with high levels

of accuracy. However, they are proving less useful on an individual basis. We are currently: (1) investigating novel analysis techniques to better capture items that distinguish groups; (2) expanding from behavioral domains to cognitive domains. The next step is to work with Dr. Ganz Chockalingham to develop an app or website that can capture these domains/items in an easy to use format that can be administered in an unsupervised fashion.

Additional Work: We completed testing using CIFASD 3 test battery and the younger age range. We tested a total of 285 subjects using this test battery in San Diego (264 were originally planned) and these added subjects have already been included in analyses. We previously reported that we translated our recruitment materials into Spanish but unplanned staffing changes prohibited us from deploying these versions. We had not initially proposed to administer materials in Spanish but hope to do so when staffing allows. Child testing will still take place in English, but this will allow us to access a larger potential subject pool. We also lowered our age limit to 5 years and have tested 19 children in this age group. The measures for the eTree are valid in this age younger range and we are using a modified version of our validation battery appropriate for younger children. We are also working on enhancing the eTree App to allow for ease of administration/data entry of the dysmorphology exam and hope to add the FONS enhancements as they come on line.

Plans for Next Funding Year: During the next reporting period, we plan to continue our efforts in meeting all our aims.

Aim 1a: we are examining why the ARND agreement is so low and hope to find a solution that optimizes our ability to accurately identify alcohol-affected youth. We hope to incorporate novel analytic techniques (e.g., Machine Learning, Decision tree, Neural Nets, Random Forest) to accelerate dissemination of the eTree to clinical settings. We are submitting an administrative supplement for this work.

Aim 1b/c: we will continue to collect data in the existing 3 sites and add 2 additional sites.

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Papers

Doyle*, L.R., Coles, C.D., Kable, J.A., May, P.A., Sowell, E.R., Jones, K.L., Riley, E.P., **Mattson**, S.N., and the CIFASD (2019). Relation between adaptive function and IQ among youth with histories of heavy prenatal alcohol exposure. Birth Defects Research, in press. Special issue on Fetal Alcohol Spectrum Disorders.
Available online 04 February 2019.
doi: 10.1002/bdr2.1463
PMCID: In Process. NIHMSID: 1013291

Doyle*, L.R., Moore, E.M., Coles, C.D., Kable, J.A., Sowell, E.R., Wozniak, J.R., Jones, K.L., Riley, E.P. **Mattson**, S.N. and the CIFASD (2018). Executive functioning correlates with communication ability in youth with histories of heavy prenatal alcohol exposure. Journal of the International Neuropsychological Society, in press. Available online 16 October 2018.
doi: 10.1017/S1355617718000772
PMCID: In Process
https://www.ncbi.nlm.nih.gov/pubmed/30322415

Suttie, M., Wozniak, J.R., Parnell, S.E., Wetherill, L., **Mattson**, S.N., Sowell, E.R., Kan, E., Riley, E.P., Jones, K.L., Coles, C.D., Foroud, T., Hammond, P., and the CIFASD (2018). Combined face-brain morphology and associated neurocognitive correlates in fetal alcohol spectrum disorders. Alcoholism: Clinical and Experimental Research, 42 (9): 1769-1782.
doi: 10.1111/acer.13820
PMCID: PMC6120799
https://www.ncbi.nlm.nih.gov/pubmed/29935097

Hendrickson, T.J., Mueller, B.A., Sowell, E.R., **Mattson**, S.N., Coles, C.D., Kable, J.A., Jones, K.L., Boys, C.J., Lee, S., Lim, K.O., Riley, E.P., & Wozniak, J.R. (2018). Two-year cortical trajectories are abnormal in children and adolescents with prenatal alcohol exposure. Developmental Cognitive Neuroscience, 30, 123-133.
doi: 10.1016/j.dcn.2018.02.008
PMCID: PMC5949095
https://www.ncbi.nlm.nih.gov/pubmed/29486453

Gross*, L.A., Moore, E.M., Wozniak, J.R., Coles, C.D., Kable, J.A., Sowell, E.R., Jones, K.L., Riley, E.P., Mattson, S.N. and the CIFASD (2018). Neural correlates of verbal memory in youth with heavy prenatal alcohol exposure. Brain Imaging and Behavior, 12 (3): 806-822. Available online 27 June 2017.
doi: 10.1007/s11682-017-9739-2
PMCID: PMC5745322
https://www.ncbi.nlm.nih.gov/pubmed/28656347

Book Chapters

Mattson, S.N., Doyle*, L.R., Glass*, L. (2019, submitted). Fetal Alcohol Spectrum Disorders and Other Teratogenic Conditions. To appear in the APA Handbook of Intellectual and Developmental Disabilities.

Doyle*, L.R. and **Mattson**, S.N. (2019). Neurobehavioral Disorder Associated with Prenatal Alcohol Exposure. In T.H. Ollendick, S.W. White, B.A. White (Eds.), The Oxford Handbook of Clinical Child and Adolescent Psychology (pp. 594-610). New York: Oxford University Press.
doi: 10.1093/oxfordhb/9780190634841.013.39

Conference Presentations

Doyle*, L.R., Chambers, C.D., Jones, S.N., **Mattson**, S.N., and the CIFASD. (2018). Validation of a decision tree for clinical identification of children affected by prenatal alcohol exposure in a low-risk sample. Presented at the Research Society on Alcoholism meeting, San Diego, June 2018. doi: 10.1111/acer.13747, Published online 31 May 2018. https://onlinelibrary.wiley.com/doi/full/10.1111/acer.13747 Inkelis, S.M., Moore, E.M., **Mattson**, S.N., Riley, E.P. (2018). Predicting prenatal alcohol exposure histories using measures of attention and activity. Presented at the Research Society on Alcoholism meeting, San Diego, June 2018. doi: 10.1111/acer.13747, Published online 31 May 2018. https://onlinelibrary.wiley.com/doi/full/10.1111/acer.13747 Carvalho, R., **Mattson**, S.N., Riley, E.P., Moore, E.M. (2018). Cognition measured with NIH Toolbox in children with histories of heavy prenatal alcohol exposure. Presented at the Research Society on Alcoholism meeting, San Diego, June 2018. doi: 10.1111/acer.13747, Published online 31 May 2018. https://onlinelibrary.wiley.com/doi/full/10.1111/acer.13747 Wetherill, L., **Mattson**, S.N., Foroud, T., Goodlett, C., and the CIFASD. (2018). Effect of prenatal alcohol exposure and parental alcohol dependence on risk of externalizing disorders in COGA and CIFASD samples. Presented at the Research Society on Alcoholism meeting, San Diego, June 2018. doi: 10.1111/acer.13747, Published online 31 May 2018. https://onlinelibrary.wiley.com/doi/full/10.1111/acer.13747

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	Seewald PM, De Jesus SY, Graves LV, Moreno CC, Mattson SN, Gilbert PE. Age-related differences on a new test of temporal order memory for everyday events. Neuropsychology, development, and cognition. Section B, Aging, neuropsychology and cognition. 2018 May;25(3):319-332. PubMed PMID: 28270013; PubMed Central PMCID: PMC5935107.
Complete	Suttie M, Wozniak JR, Parnell SE, Wetherill L, Mattson SN, Sowell ER, Kan E, Riley EP, Jones KL, Coles C, Foroud T, Hammond P. Combined Face-Brain Morphology and Associated Neurocognitive Correlates in Fetal Alcohol Spectrum Disorders. Alcoholism, clinical and experimental research. 2018 September;42(9):1769-1782. PubMed PMID: 29935097; PubMed Central PMCID: PMC6120799.
Complete	Doyle LR, Moore EM, Coles CD, Kable JA, Sowell ER, Wozniak JR, Jones KL, Riley EP, Mattson SN. Executive Functioning Correlates With Communication Ability in Youth With Histories of Heavy Prenatal Alcohol Exposure. Journal of the International Neuropsychological Society : JINS. 2018 November;24(10):1026-1037. PubMed PMID: 30322415; PubMed Central PMCID: PMC6237635.
In Process at NIHMS	Doyle LR, Coles CD, Kable JA, May PA, Sowell ER, Jones KL, Riley EP, Mattson SN. Relation between adaptive function and IQ among youth with histories of heavy prenatal alcohol exposure. Birth defects research. 2019 February 4. PubMed PMID: 30719847.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Nothing to report

C.3 TECHNOLOGIES OR TECHNIQUES

Category	Explanation
Other	https://fasdtree.org This website accompanies the eTree app that was developed as part of this project.

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period?

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization?

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Category	Explanation
Other	Book Chapters Mattson, S.N., Doyle*, L.R., Glass*, L. (2019, submitted). Fetal Alcohol Spectrum Disorders and Other Teratogenic Conditions. To appear in the APA Handbook of Intellectual and Developmental Disabilities.

	Doyle*, L.R. and Mattson, S.N. (2019). Neurobehavioral Disorder Associated with Prenatal Alcohol Exposure. In T.H. Ollendick, S.W. White, B.A. White (Eds.), The Oxford Handbook of Clinical Child and Adolescent Psychology (pp. 594-610). New York: Oxford University Press. doi: 10.1093/oxfordhb/9780190634841.013.39
Other	Conference Presentations Doyle, L.R., Chambers, C.D., Jones, S.N., Mattson, S.N., and the CIFASD. (2018). Validation of a decision tree for clinical identification of children affected by prenatal alcohol exposure in a low-risk sample. Presented at the Research Society on Alcoholism meeting, San Diego, June 2018. doi: 10.1111/acer.13747, Published online 31 May 2018. https://onlinelibrary.wiley.com/doi/full/10.1111/acer.13747 Inkelis, S.M., Moore, E.M., Mattson, S.N., Riley, E.P. (2018). Predicting prenatal alcohol exposure histories using measures of attention and activity. Presented at the Research Society on Alcoholism meeting, San Diego, June 2018. doi: 10.1111/acer.13747 Carvalho, R., Mattson, S.N., Riley, E.P., Moore, E.M. (2018). Cognition measured with NIH Toolbox in children with histories of heavy prenatal alcohol exposure. Presented at the Research Society on Alcoholism meeting, San Diego, June 2018. doi: 10.1111/acer.13747, P Wetherill, L., Mattson, S.N., Foroud, T., Goodlett, C., and the CIFASD. (2018). Effect of prenatal alcohol exposure and parental alcohol dependence on risk of externalizing disorders in COGA and CIFASD samples. Presented at the Research Society on Alcoholism meeting, San Diego, June 2018. doi: 10.1111/acer.13747

Mattson U01 Decision Tree (eTree) and Neurobehavior	Current Month	Cumulative Total	May 2019 Goal	% to Goal	Overall Goal	Goal Date	May 2018 Goal	May 2019 Goal	May 2020 Goal	May 2021 Goal	May 2022 Goal
Decision Tree (eTree) - Aim 1b											
San Diego (UCSD and SDSU)	13	147	100	147.0%	260	5/31/2022	40	100	160	220	260
Minnesota	0	35	90	38.9%	90	5/31/2019	45	90	90	90	90
San Diego Psych Clinic [YRS 3 & 4]	YR3	YR3	0	YR3	50	5/31/2021	0	0	25	50	50
San Diego Dev'l BX Peds [YRS 3 & 4]	YR3	YR3	0	YR3	50	5/31/2021	0	0	25	50	50
NB Battery - Aim 1c/2c											
San Diego	3	67	45	148.9%	120	5/31/2022	15	45	75	105	120
Minnesota	1	56	60	93.3%	90	5/31/2020	30	60	90	90	90
Online FONS YRS 2-5 Aim 2a											
WebPortal FONS Completed	YR3	YR3	0	YR3	2000	5/31/2022	0	0	667	1333	2000

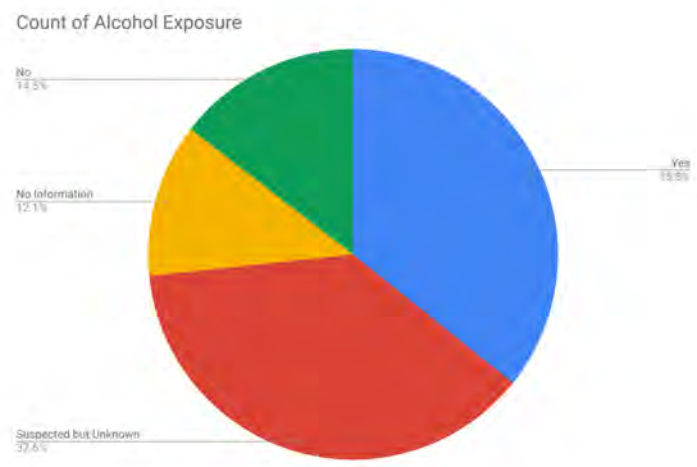
Cumulative - at the end of each year.

	Start	End
Current month (defined by project) =	1/1/2019	1/31/2019
Date of project numbers update entry =	2/21/2019	

FYI:

eTree Validation & Subjects - Aim 1a	Available	Received	Status
CoFASP San Diego - AE, CON	994	896	Analysis in progress.
CIFASD Ukraine - AE, CON	250	0	Will request once CoFASP sample is analyzed to use as a validation sample.

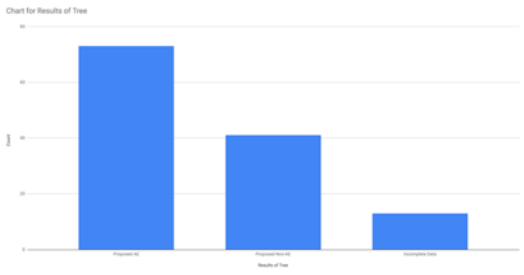
Subjects Collected Using eTree 12/18/18



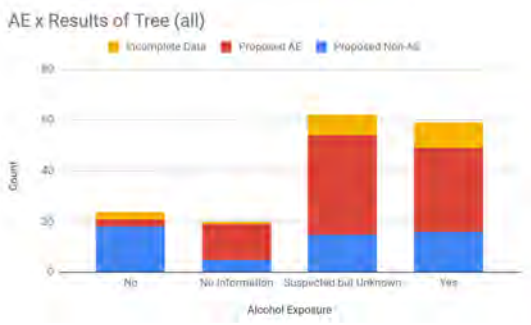
	N	%
Total	165	100
White	107	64.85
Hispanic/Latino	46	27.88
Female	49	29.70

Mid-Year Update

Results of Tree for All Subjects



Results of Tree by Alcohol Exposure



Mid-Year Update

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Aim 1. Use strain-specific differences in ethanol sensitivity to characterize modifiers of FASD. Strain-specific differences in ethanol sensitivity are an excellent source to identify genetic signatures that modify ethanol teratogenesis. However, our ability to identify these signatures of differentially expressed genes and characterize which are functionally relevant in the response to alcohol has been limited. State of the art high-throughput whole transcriptome sequencing (RNA-Seq) is a non-biased approach to comprehensively identify differential gene expression. Bioinformatic analyses, such as Weighted Gene Correlation Network Analysis, provide ways to identify gene modules and hub genes within these modules likely to be of central importance in a genetic pathway. Recent advances in gene editing via CRISPR/Cas9 in zebrafish have generated a highly efficient method for rapidly testing gene function, allowing us to go from large gene datasets to a functional understanding of differentially regulated genes. For this Aim, we will perform RNA-seq analyses of developing brain and face tissue from gastrulation and neurulation-stage mouse embryos, with comparisons being made between stage-matched alcohol-exposed and control samples from alcohol resistant and sensitive sub-strains of mice. We will A) Identify differentially expressed genes in ethanol-sensitive versus resistant mouse strains that underlie ethanol teratogenesis B) Rapidly determine the function of differentially expressed genes in ethanol teratogenesis and C) Use mouse genetics in conjunction with Peter Hammond's face and brain analyses to determine the facial, neural and neurobehavioral consequences of gene-ethanol interactions.

Aim 2. Employ screening approaches to identify and confirm modifiers of gene-ethanol interactions. As a complex phenotype, FASD is likely to be the concerted result of complex multifactorial interactions. It is particularly likely that the interaction of multiple genetic loci regulating ethanol sensitivity and resistance govern the overall outcome of an ethanol exposure. Several of the ethanol-sensitive mutants that we have identified are haploinsufficient in the presence, but not absence, of ethanol. These dominant effects of ethanol, with the high fecundity of zebrafish, can be used to identify further genetic modifiers of ethanol teratogenesis. Additionally, whole exome sequencing in humans (PI Foroud) is likely to identify multiple loci implicated in human FASD. Using zebrafish genetics with follow up analyses in mouse, we will A) Use forward genetics to identify and characterize genetic suppressors of ethanol teratogenesis, B) Determine the function of suppressor mutations in the genesis of ethanol teratogenesis in mouse, and C) Utilize gene editing approaches to examine the role of variants identified in the human studies.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: B2-Accomplishments-2019a.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

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B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Aim1: During the next period, we will continue to use zebrafish, CRISPR/Cas9, as well as rapamycin to further refine our candidate gene list obtained from our 6J/6N baseline and ethanol response RNA-Seq data. We will continue to analyze the ethanol-response RNA-Seq data, as well as new RNA-Seq data in Bax knockouts to further define ethanol's mechanism of action and more candidate genes to be tested in zebrafish. Aim 2 The modifier screen will continue as the human genetic anslyses (Foroud) begins to identify candidate genes. Based on our previous data from Aim 1, we are continuing to explore the role of the p53 pathway in ethanol's teratogenesis, as well as the genes Efcab7 (we have obtained the mice) and Kif2a, and other genes identified in the various aspects of this project.

B.2 What was accomplished under these goals?

1) Major Activities. Aim 1: In this reporting period we have utilized RNA-Seq to explore the differential responses to ethanol between embryos from an ethanol-sensitive strain of mice (6J) and embryos from an ethanol-resistant strain (6N). To do this, we micro-dissected the rostral portions of gestational day (GD) 7.25 (6 hrs following the beginning of ethanol exposure) and GD 7.5 (12 hrs after the beginning of ethanol exposure) embryos (the area that will give rise to the brain and face – structures that are affected by ethanol exposure at this period). Following RNA isolation, whole transcriptomic profiling was performed for the 7.25 hr time point (7.5 hr time point is ongoing), providing a complete picture of gene expression in each strain of mouse and how they respond to ethanol. Bioinformatic analyses was performed to reveal individual differentially expressed genes (DEG), followed by a detailed examination of the function of each of these genes, as well as their potential role in development. Further Ingenuity Pathway Analysis (IPA) was performed to explore genetic pathways that are differentially expressed between the two strains of mice in both controls and ethanol treated embryos. We selected 7 top candidate genes for mutant analyses in zebrafish: *dynlt1, efcab7, fam65b, kit, nnt, wdfy1* and *wt1a*.

Aim 2: We have raised a stock of mutagenized zebrafish with which to perform our analyses. We have also performed dose response analyses with a Pdgf receptor inhibitor and rapamycin (an inhibitor of mTOR). We will use these inhibitors instead of *pdgfra* mutants to speed our analyses. Finally, we have begun testing candidate genes in knockout mouse strains that will examine the role of individual genes (e.g. *p53*, *Mns1*) identified by our pathway analyses in modifying susceptibility to prenatal ethanol exposure. The study involving p53 is ongoing, although preliminary analyses suggests that deleting the p53 gene protects against ethanol teratogenicity. Deleting Mns1 exacerbates the effects of ethanol; these data have been published (Boschen et al., 2018; PMID: 30129265).

2) Specific Objectives. Aim 1: Identify genes and pathways that are differentially expressed in a strain of mouse that is exquisitely sensitive to prenatal alcohol exposure (6J) and a strain that is relatively resistant (6N). Aim 2: Identify genes and pathways that modify the teratogenic effect of ethanol on *pdgfra* heterozygotes.

3) Significant Results. Aim 1: At 6 hrs following ethanol exposure, 515 genes and 41 canonical pathways were significantly affected in the 6J's, as opposed to 358 genes and 11 pathways in the 6N's. The top differentially regulated pathways in the 6J's included downregulation of cell proliferation, growth factors, and second messengers, as well as upregulation of the Wnt and the HIPPO pathways. Decreased expression of pro-proliferation genes and increased expression of pro-apoptotic pathways could have significant impacts on the growth trajectories of face and brain tissue in the 6J's. In the 6N's, PAE resulted in downregulation of RhoGDI signaling and upregulation of cell motility and adhesion pathways.

Aim 2 We have finalized our dose response analyses and are currently testing doses of Pdgfr inhibitor IV and rapamycin that do not result in significant defects on our mutant stocks.

4) Key Outcomes and Other Achievements. Based on the results obtained above in the mouse component (Parnell Lab), the zebrafish component (Eberhart Lab) has obtained oligos to generate gRNAs in order to make CRISPR-based mutations in 7 top priority genes. We currently have stocks for all of these genes. We have preliminary data showing that *nnt* and *dynlt1* mutants are sensitive to ethanol. These genes will be further tested in human populations (Foroud). These initial experiments in mice will also set the baseline for ongoing experiments exploring how these two strains of mice respond to an acute alcohol exposure, which will both identify more candidate genes regulating sensitivity to prenatal alcohol exposure, and also suggest mechanistic pathways involved in ethanol's teratogenesis. Furthermore, experiments are now beginning to characterize transcriptomic differences between Bax wild-type (highly susceptible) and knockout (non-susceptible) embryos.

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

Dr. Karen Boschen, a third-year post-doctoral fellow in the Parnell Lab who has been working on this project, has been using these experiments to further her knowledge of embryology, RNA-Seq technologies, bioinformatics analyses, and genetic pathway functions. Dr. Boschen's increased knowledge and skill in these areas has assisted her in obtaining an NIH Postdoctoral Individual National Research Service Award (F32) from NIAAA, that began in March 2018. As a part of this training, and as a routine part of UNC's post-doctoral requirements, we have an annual review and Individual Development Plans for each post-doctoral fellow. In the next year, Dr. Boschen will submit a K99/R00 proposal to NIAAA.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	Suttie M, Wozniak JR, Parnell SE, Wetherill L, Mattson SN, Sowell ER, Kan E, Riley EP, Jones KL, Coles C, Foroud T, Hammond P. Combined Face-Brain Morphology and Associated Neurocognitive Correlates in Fetal Alcohol Spectrum Disorders. Alcoholism, clinical and experimental research. 2018 September;42(9):1769-1782. PubMed PMID: 29935097; PubMed Central PMCID: PMC6120799.
Complete	Boschen KE, Gong H, Murdaugh LB, Parnell SE. Knockdown of Mns1 Increases Susceptibility to Craniofacial Defects Following Gastrulation-Stage Alcohol Exposure in Mice. Alcoholism, clinical and experimental research. 2018 November;42(11):2136-2143. PubMed PMID: 30129265; PubMed Central PMCID: PMC6214710.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Nothing to report

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period?

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization?

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

Workshop chair:

J.K Eberhart. Zebrafish models of gene-environment interactions. International Zebrafish Meeting, Madison, WI. June 20-23.

Abstracts:

T. Kuka and J.K. Eberhart. A novel lipoprotein receptor protects against ethanol teratogenesis. International Zebrafish Meeting, Madison, WI. June 20-23.

C.B. Lovely, M.E. Swartz, N.McCarthy, J. Norrie, T. Henegar and J.K. Eberhart. Requirement for Bmp signaling in endoderm and jaw development identified from a gene-ethanol screen in zebrafish. Society for Developmental Biology, Portland, OR, July 19-23.

T. Kuka and J.K. Eberhart. A novel lipoprotein receptor protects against ethanol teratogenesis. Society for Developmental Biology, Portland, OR, July 19-23.

A. Sidik, G.B. Dixon and J.K. Eberhart. Characterizing the early transcriptional response to ethanol teratogenesis. Society for Developmental Biology, Portland, OR, July 19-23.

Boschen KE, Eberhart JK, Parnell SE. Transcriptome-wide analysis of ethanol sensitive and insensitive mouse strains during early embryonic development. Alcohol Clin Exp Res 42(S1):42A, 2018

Boschen KE, Parnell SE. Transcriptome-wide analysis in the neural tube following mid- neurulation stage ethanol exposure in C57BL/6J mice. Alcohol Clin Exp Res 42(S1):42A, 2018.

Fish EW, Boschen KE, Leitzinger JO, Venktatasubramanian D, Parnell SE. The primary cilia gene Kif3a mediates vulnerability to the effects of neurulation stage alcohol exposure on adolescent exploratory behavior. Alcohol Clin Exp Res 42(S1):42A, 2018.

Presentations:

A. Sidik, G.B. Dixon and J.K. Eberhart. Characterizing the early transcriptional response to ethanol teratogenesis. International Zebrafish Meeting, Madison, WI. June 20-23.

J. K. Eberhart, Y. Fernandes, S. Tucker and N. McCarthy. Synergistic gene-environment interactions in a zebrafish model of Fetal Alcohol Spectrum Disorders. ISBRA, Kyoto, Japan, Sept 9-13.

Parnell, SE. Identifying genetic modifiers of susceptibility to prenatal alcohol exposure in mice. The 19th Congress of International Society for Biomedical Research on Alcoholism (ISBRA). Kyoto, Japan. Symposium.

Parnell, SE. Prenatal ethanol exposure induces a “transient ciliopathy”: A novel mechanism for ethanol’s pathogenesis. The 41st Annual Meeting of Research Society on Alcoholism, San Diego, CA. Symposium.

Update date:	2/25/2019	
Parnell Eberhart U01 Fish and Mice Genetics	**Status - % Complete**	**Goal Date**
Exp 1.1: 1st RNA-Seq data set (2/2)	100% 2 data sets completed - analysis ongoing	5/31/2018
Exp 1.2: CRISPR KO/analysis of 9/10 candidate genes	1 mutant in hand, 8 CRISPR lines generated (100%) analysis is ongoing (25% tested for ethanol sensitivity, genotyping in progress)	5/31/2018
Exp 2.1: Screen 50 mutagenized genomes for ethanol suppressor mutations	12 genomes screened (24%) - currently retooling screen	5/31/2018
Exp 1.1: 2nd RNA-Seq data set (1/2)	75% 1 data set completed-analyzing 2nd set 90% collected - had some bad rna extraction kits - recollecting 12 samples :(	11/30/2018
Exp 1.1: 3rd RNA-Seq data set (0/1)	25%	5/31/2019
Exp 1.2: CRISPR KO/analysis of 10 candidate genes		5/31/2019
Exp 2.1: Screen 50 mutagenized genomes for ethanol suppressor mutations		5/31/2019
Exp 2.2: Screen 1-2 candidate suppressors in mice	1st gene (p53) ongoing (35%)	5/31/2019
Exp 1.1: 4th RNA-Seq data set (0/2)	YR3	11/30/2019
Exp 1.2: CRISPR KO/analysis of 10 candidate genes	YR3	5/31/2020
Exp 1.3: Screen 1-2 gene/ethanol interactions in mice	1 gene (Mns1) finished (published), 3rd gene (Efcab7) colony growing-starting to test gene/alcohol interaction	5/31/2020
Exp 2.1: Screen 50 mutagenized genomes for ethanol suppressor mutations	YR3	5/31/2020
Exp 2.2: Screen 1-2 candidate suppressors in mice	YR3	5/31/2020
Exp 1.3: Screen 1-2 gene/ethanol interactions in mice	YR4	5/31/2021
Exp 2.1: Analysis of suppressor mutations	YR4	5/31/2021
Exp 2.2: Screen 1-2 candidate suppressors in mice	YR4	5/31/2021
Exp 2.3: CRISPR KO/analysis of 5 human genes	YR4	5/31/2021
Exp 1.3: Screen 1-2 gene/ethanol interactions in mice	YR5	5/31/2022
Exp 2.1: Analysis of suppressor mutations	YR5	5/31/2022
Exp 2.3: CRISPR KO/analysis of 5 human genes	YR5	5/31/2022

Experiment 1.1. Identify differentially expressed genes in ethanol-sensitive versus resistant mouse strains that underlie ethanol teratogenesis.

Experiment 1.2. Rapidly determine the function of differentially expressed genes in ethanol teratogenesis.

Experiment 1.3. Use mouse genetics to determine the facial, neural and neurobehavioral consequences of gene-ethanol interactions

Experiment 2.1. Use forward genetics to identify and characterize genetic suppressors of ethanol teratogenesis.

Experiment 2.2. Determine the function of suppressor mutations in the genesis of ethanol teratogenesis in mouse.

Experiment 2.3. Utilize gene editing approaches to examine the role of variants identified in the human studies.

Baseline strain comparison

Decreased cell proliferation and increased apoptosis pathways

Numerous large differences in several cilia genes (e.g. Dynlt1 & Efcab7)

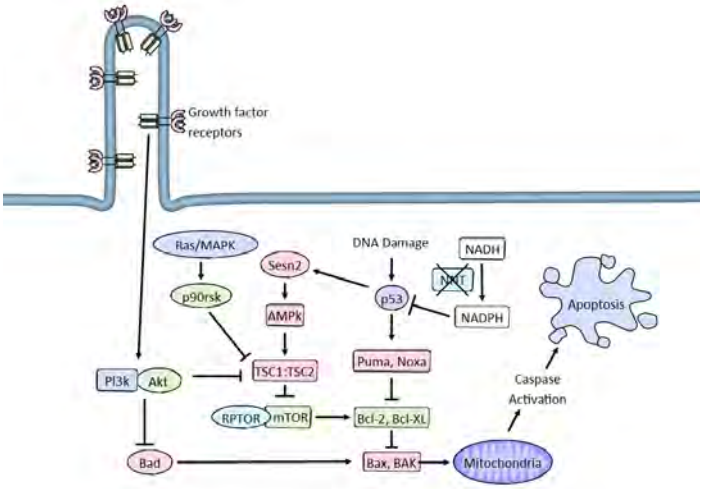
Immediate (6hr) response to alcohol

Amplification of abnormal cell proliferation and apoptosis processes in 6J mice

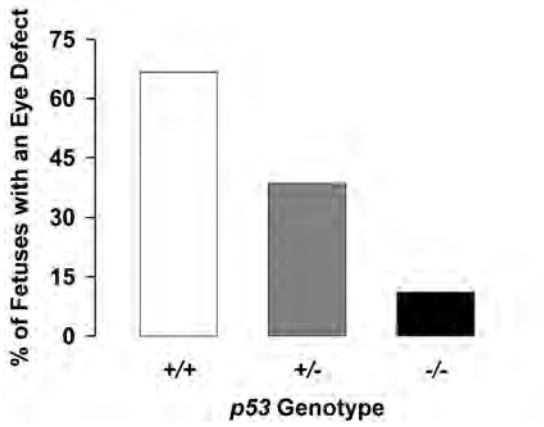
Altered cell migration pathways in 6N mice

Intermediate (12 hr) response to alcohol

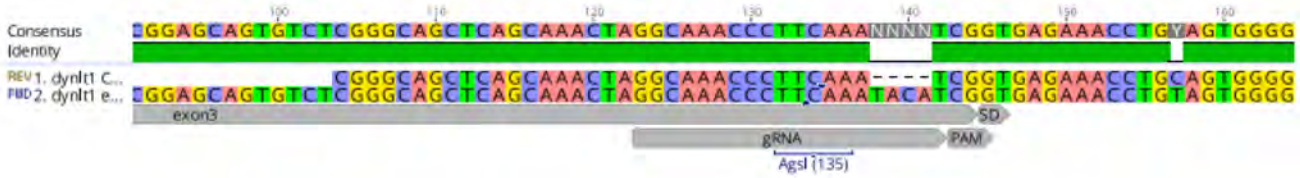
100% collected – will be sequenced after winter break



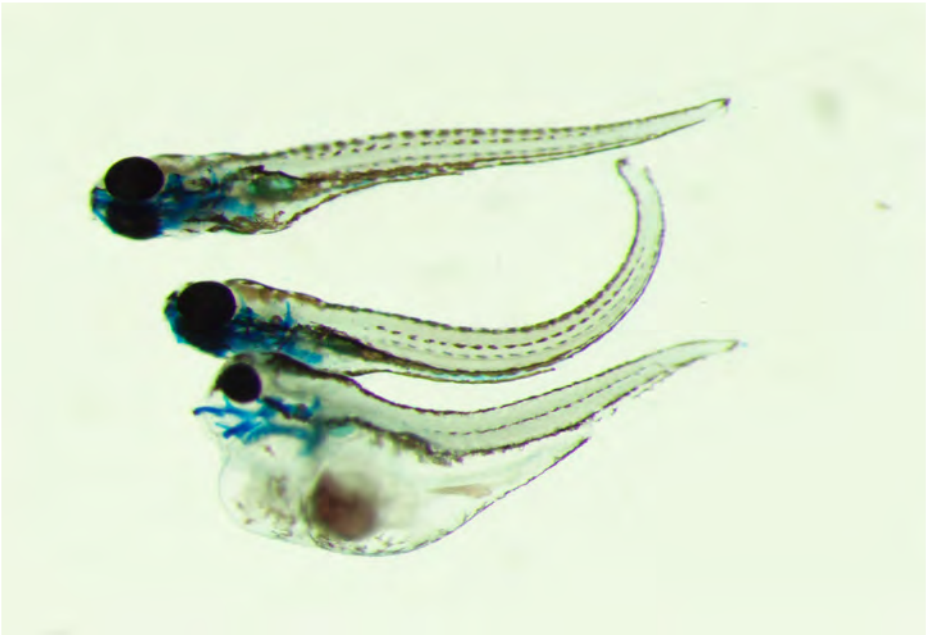
Preliminary analysis of p53



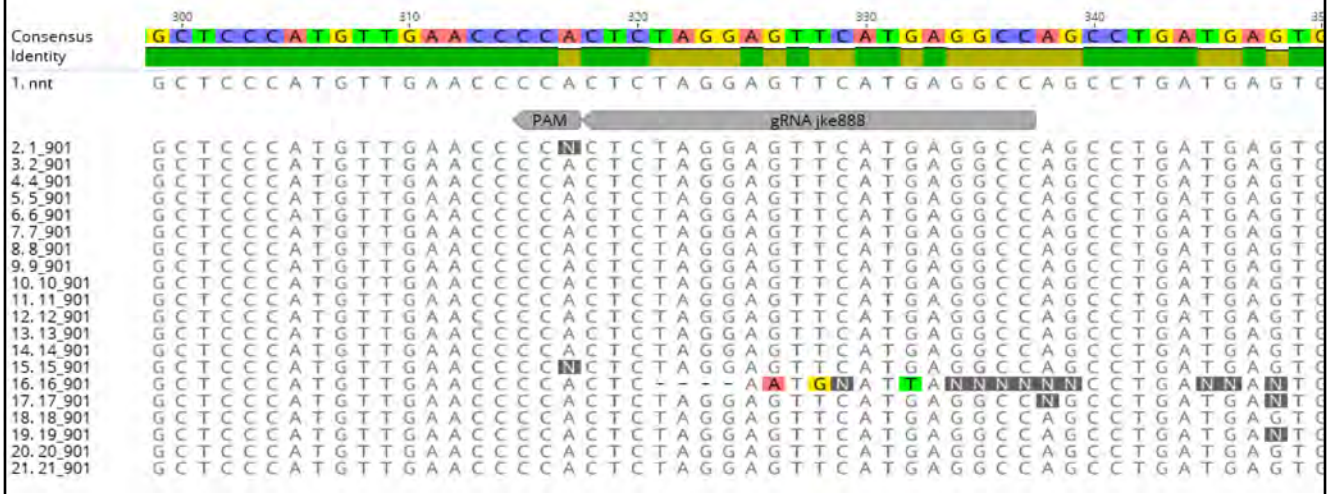
A CRISPR-induced frame-shift mutation in *dynlt1*



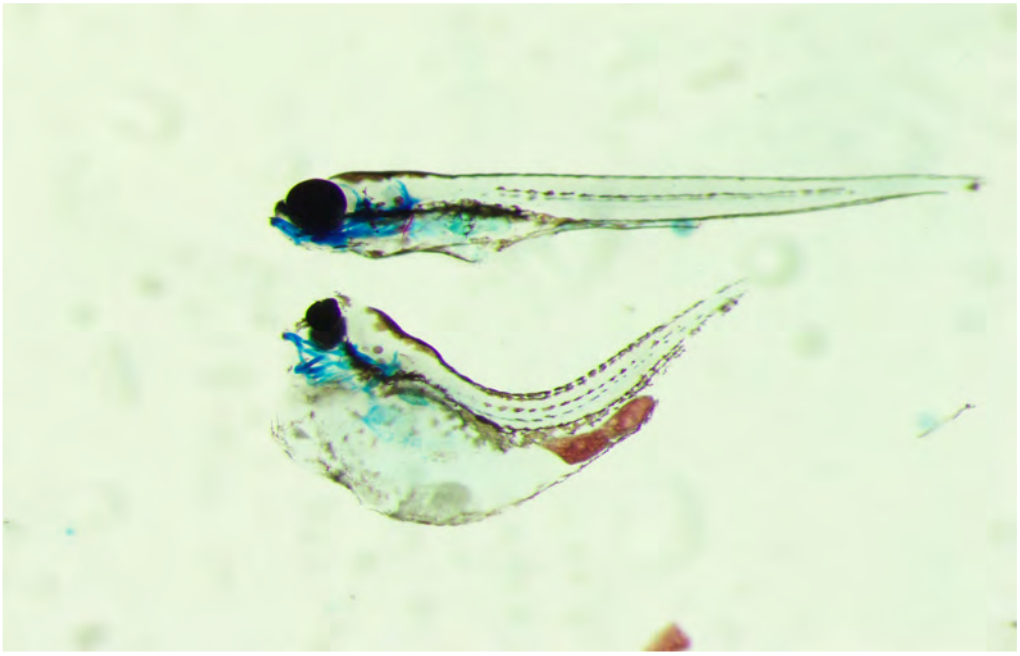
Potential ethanol-induced defects in *dynlt1* heterozygotes



Frame-shift mutation in *nnt*



Possible ethanol-induce *nnt* haploinsufficiency



B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Aim 1. Development of “FMF Connect:” Develop a novel Cloud-based mHealth app designed for caregiver use, derived from our work on the scientifically validated FMF Program for caregivers raising children with FASD, and building on our existing framework for the development of medical apps.
Aim 1a. Identify and refine functionalities required to efficiently address the needs of children with FASD and their families/caregivers.
Aim 1b. Develop Android and iOS versions of the mHealth app.

Aim 2. Feasibility Study: Using qualitative and quantitative methodologies, assess the feasibility and users’ satisfaction and experiences with the innovative app-based mHealth intervention program built in Aim 1 with a diverse group of 30 families raising children (ages 3-12) with FASD.

Aim 3. Hybrid Implementation-Effectiveness Study: Examine how caregiver use of FMF Connect relates to caregiver and child outcomes in the context of a randomized controlled trial (RCT; N=120) comparing intervention and waitlist groups.
Aim 3a. Identify patterns of app usage (i.e., frequency, duration, use of core components) that relate to greater improvements in child and caregiver outcomes using machine learning techniques.
Aim 3b. Examine whether families randomized to FMF Connect have improved outcomes relative to the waitlist group at 3-months and 6-months after baseline.
Aim 3c. Examine whether parental attributions of child misbehavior mediate intervention-related changes in parenting efficacy and child behavior.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

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B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

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B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

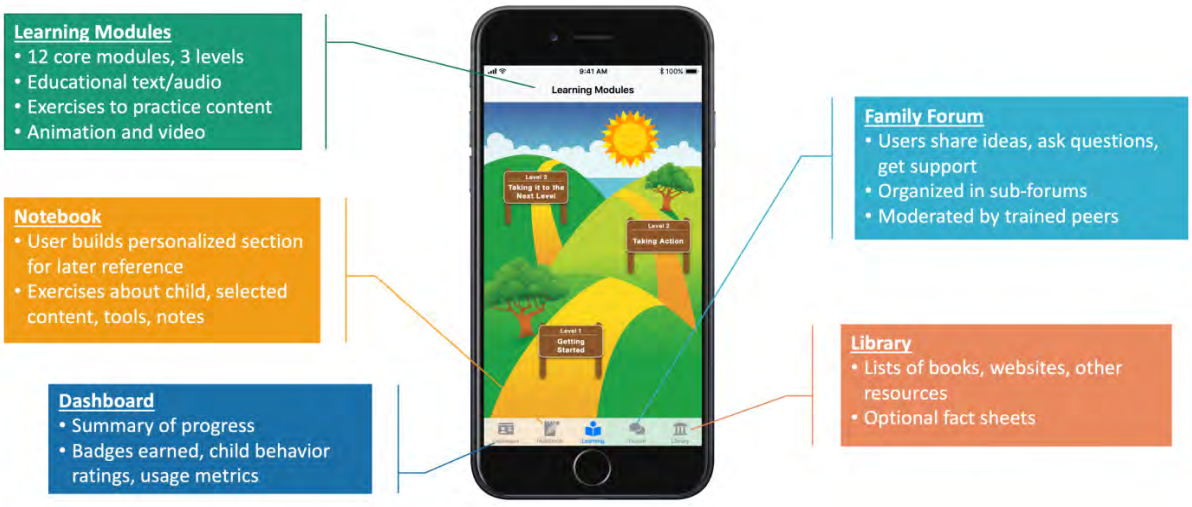
Over the next funding period in Year 3 of this project, we will complete Aim 2. We will complete systematic analysis of focus group and interview data collected from beta-testers and will use these findings to inform refinements to the app. A manuscript is planned summarizing these findings.

We will then complete the planned feasibility trial (n=30) over a 3-month period. We will trial pre- and post-intervention quantitative measures collected online with each participant and will also complete qualitative interviews. We will hire and train a full-time research assistant to facilitate this work. A manuscript is also planned to present results from this trial. Findings from this trial will guide any further refinements to the app, as well as inform recruitment and measurement methodologies for the subsequent RCT planned for years 4 and 5.

B.2 ACCOMPLISHMENTS

1) Major Activities. Over the second year of the project, we have accomplished the following activities:

- We are on target to complete the initial FMF Connect app prototype on schedule for February 28th, 2019. Figure 1 illustrates the 5 primary app components and interface design of the Learning Modules for reference. Key activities completed this year are as follows:
    - A third round of editing was completed for all Learning Module content to optimize the phone interface after programming was complete.
    - Over 55 hours of filming was completed with families raising children with FASD to provide video examples to aid in teaching users key content in the Learning Modules of the app. All video was reviewed and edited into planned sequences for the app. Preliminary voiceover was recorded for sequences that will be used in beta-testing. A professional voiceover artist was hired who will record final voiceovers following beta-testing feedback.
    - Hired a graphic artist to develop and animate 4 characters that will be used in the app to perform specific educational and motivational functions.
    - Drafted and edited key content for the Library in the app. The Library will continue to grow over the course of the project.
    - Transitioned from Google Cloud platform to Amazon Web Services for better integration and full HIPAA compliance.
    - Improved the implementation of the functionalities developed in Year 1 and carefully organized the different elements of the app (content, visual interfaces and interface controllers) to facilitate future app updates and refinements as well as development of new components.
    - Continued refinement of the user interface and functionalities in response to participant feedback from focus groups.
    - To speed up the development and maximize the quality of the initial prototype, priority has been given to the iOS version of the app. Implementation of the Android version will continue during the beta-testing of the iOS version, and will take advantage of the beta-testers' feedback.



- Completed 2 additional focus groups in June in San Diego and Los Angeles, for a total of 7 focus groups (3 Rochester, 1 Atlanta, 1 Minneapolis, 1 San Diego, 1 Los Angeles). Focus groups elicited participants' perspectives on aspects such as the interface design, ease of use, relevance of components and content, and barriers and facilitators of use. We enrolled a total of 25 participants across focus groups. Support from other CIFASD investigators (Coles, Wozniak, Riley, Mattson, Jones, Mitchell) was invaluable in recruitment and facilitating logistics for focus group meetings.

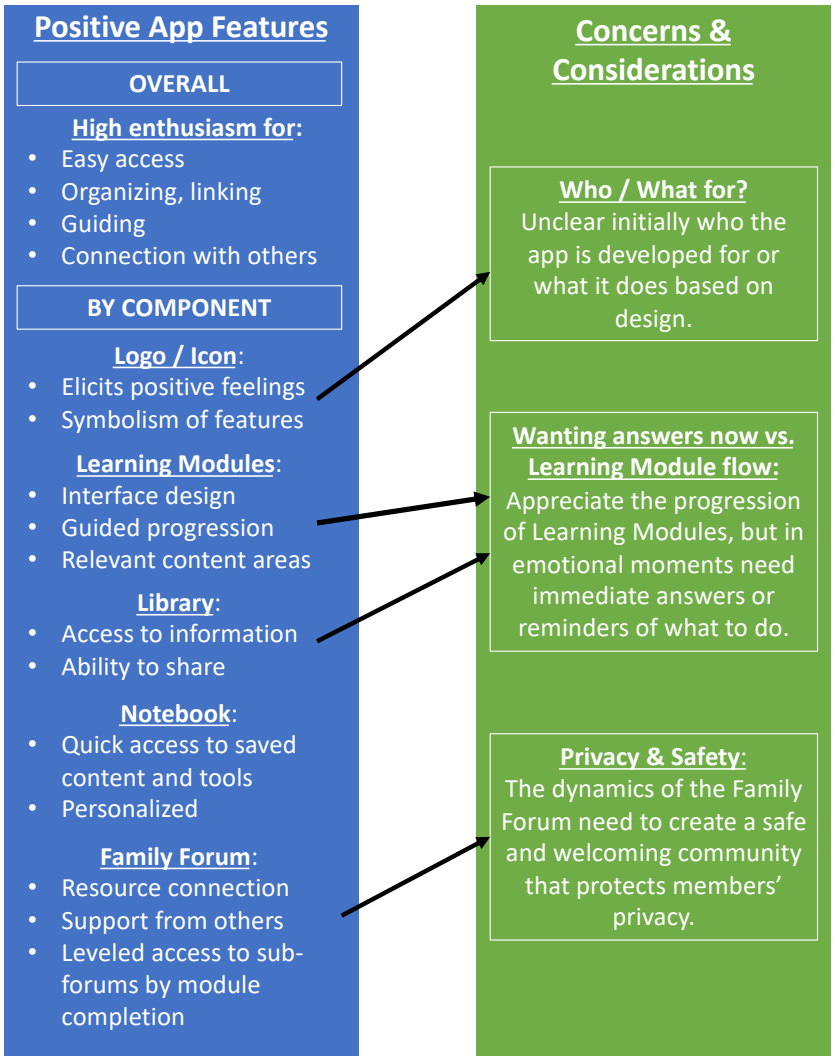
- Audio recordings from focus groups were transcribed verbatim and were analyzed using systematic qualitative methods, supported by Atlas.ti software. Results from these analyses are reported below. A manuscript presenting these findings is in preparation, under the lead of Dr. Petrenko.
- An additional manuscript is in preparation under the direction of Dr. Tapparello discussing the methods and tools used to adapt a therapist-based parent consultation program to a mobile health platform. This manuscript will also highlight the technical requirements and difficulties of developing this type of mHealth app.
- Three abstracts were selected for upcoming presentations at conferences. We will be presenting at the 8th International Conference on FASD in Vancouver Canada on March 6-9th, 2019. We have a presentation as part of the CIFASD plenary on Friday March 8th, followed by a 90-min symposia where we will be able to present the prototype of the FMF Connect app and discuss our focus group findings in detail. We also just learned that our third abstract was accepted for a symposia organized by CIFASD at the Research Society on Alcoholism in June 2019.
- We have initiated recruitment for beta-testing of the app prototype which is currently scheduled for mid-late March. We will be recruiting parents of children with FASD and service providers to try out the app and provide feedback. Beta-testing is scheduled to last approximately 6 weeks. Another round of focus groups will be completed in May.

2) Specific Objectives. Aim 1 is being accomplished in Years 1 and 2 of the grant. Aim 2 will be completed in Year 3, and Aim 3 will be conducted in Years 4-5.

The specific objectives of Aim 1 are to 1) identify and refine the functionalities required to address the needs of children with FASD and their families on important outcomes, and 2) develop Android and iOS versions of the FMF Connect app.

As detailed above, we are on target to complete the initial prototype of the FMF Connect app on schedule. Focus group findings have been instrumental in refining the app functionalities and are summarized in the following section. As mentioned above, we are also actively recruiting for beta-testing in March-April, followed by an additional round of focus groups and interviews. Results from this round of focus groups will aid in additional refinements to the app in preparation for our Feasibility trial scheduled in Year 3 and larger RCT in Years 4-5.

3) Significant Results. Figures 2 and 3 summarize the findings that garnered the most enthusiasm and discussion during focus groups. These are the themes that were most important to participants. The left-hand column in Figure 2 (blue) illustrates the features of the FMF Connect app that were viewed most favorably. Participants emphasized the interconnections between app components and the ease of access to important content and support from other parents. The right-hand column of Figure 2 (green) summarizes three main concerns or considerations raised by participants. Participants grappled with these topics in group discussion. Opinions were mixed or evolved over the course of the discussion. A number of suggestions were offered about how to respond to these considerations.



Consistent with our prior work, participants spoke with strong emotion about the systems barriers they face in obtaining supports and services for their children. The left side of Figure 3 summarizes how participants perceived ways the FMF Connect app could help families address some of these systems barriers. The right side includes areas where participants want additional focus, either within or adjunct to the app. The desire to share information with providers, family members, and the individual with FASD was frequently raised. The goal was often stated as helping them understand the experience of the person with FASD in order to better support them.

App Addresses Systems Barriers	Needs That Remain
Limited access to FASD-informed care: • Access content and support easily in the app • Can learn about available supports from other families in the Forum • Learn advocacy skills in app to help access community supports • The ability to share information from the app with providers to increase their knowledge about FASD care	**Recommended Within FMF Connect:** • An up to date resource directory of FASD-informed providers and community resources • Component or resources to use with children **Adjunct or Separate Apps:** • Apps and educational resources designed for providers • Apps for adolescents / adults and their caregivers

4) Key Outcomes and Other Achievements. We are on target for the completion for the initial app prototype and are excited to begin beta-testing. The beta-testing process will continue our iterative and systematic

development of the app, involving soliciting input from key stakeholders, and refining functionalities. This will result in a rigorously designed app that has high potential for success in meeting family needs and improving outcomes, as will be documented in subsequent trials in aims 2 and 3. The systematic, qualitative research findings that will result from this study can also inform the development of other FASD interventions and promote systematic, rigorous intervention research in the field.

B.4 Training & Professional Development

We have had the opportunity to include several additional undergraduate and graduate students this year as part of the project. All of these students were new to the field of FASD and through their experiences on the project have advanced their scientific and professional development skills.

During the summer of 2018, two undergraduate students in psychology completed summer internships with Dr. Petrenko and learned how to transcribe and begin preliminary coding of qualitative data collected from the focus groups. They also assisted in developing animated video sequences for the app.

Two undergraduate students in engineering were awarded competitive summer fellowships at the University of Rochester to work with Dr. Tapparello on this project. The students completed initial programming of the learning modules in Andorid and presented their work at a symposia. One of these students, Jordan Floyd, has continued working on this project during the academic year and is advancing in her knowledge of programming and mHealth interventions.

A graduate student under the mentorship of Dr. Petrenko has also joined the team. Carson Kautz is a 1st year graduate student in clinical psychology. Ms. Kautz attended a 6-day training led by Dr. Petrenko on the standard Families Moving Forward (FMF) Program over the summer. A deep understanding of this program has facilitated Ms. Kautz's contributions to content development and team discussions on app functionalities. She is also beginning her master's project that is focusing on developing implicit and explicit measurement of caregiver attributions ("reframing" in FMF). These measures will be used in the planned feasibility trial and RCT.

Dr. Tapparello has been engaged in self-study of different open source tools and libraries for app development.

CIFASD Interactions:
The overall mission of CIFASD is to inform and develop effective interventions for FASD. A common theme across multiple projects and resources is to increase capacity and accessibility for screening, diagnosis, and intervention for FASD. An app-based intervention is a logical step for systematic CIFASD aims and has potential to reduce significant barriers to families accessing evidence-based information and support.

This project benefits from the shared resources and capacities offered by CIFASD, permitting this intervention research to be conducted on a larger scale than might otherwise be possible as a stand-alone effort. Specifically, we have benefitted this year from interactions with other CIFASD investigators and resources (i.e., Riley, Mattson, Coles, Mitchell, Wozniak, Jones) who have assisted with recruitment efforts and logistics for focus groups. In turn, we have also assisted with recruitment for Dr. Foroud's human genetics project by informing patients in our clinic and family support groups. We have also offered to collect data in coordination with a separate intervention trial in my lab for Drs. Foroud and Suttie, with the assistance of the admin core who facilitated obtaining necessary equipment (3D camera).

Inclusion Enrollment Report

Inclusion Data Record (IDR) #: 174020

Using an Existing Dataset or Resource: No

Delayed Onset Study ?: No

Clinical Trial: Yes

Enrollment Location: Domestic

NIH Defined Phase III Clinical Trial: No

Study Title: Development and Evaluation of an Evidence-Based Mobile Health Caregiver Intervention for FASD

Planned Enrollment

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/Alaska Native	1	0		0	0					**1**
Asian	5	1		0	0					**6**
Native Hawaiian or Other Pacific Islander	0	0		0	0					**0**
Black or African American	12	4		3	1					**20**
White	91	14		11	2					**118**
More than One Race	3	1		1	0					**5**
Unknown or Not Reported										
Total	**112**	**20**		**15**	**3**					**150**

Cumulative Enrollment

NOTE: No cumulative enrollment data exists. Although prompted to do so, the PD/PI did not enter information. No data can be displayed.

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Nothing to report

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period?

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization?

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

Manuscripts

Petrenko CLM, Parr J, Kautz C, & Tapparello C (in preparation). Caregiver perspectives on the design and development of a mobile health intervention for FASD.

Presentations

1. Petrenko CLM & Tapparello C (2019) Families Moving Forward – Connect: Developing a Mobile Health Intervention for Families Raising Children with Fetal Alcohol Spectrum Disorders. Plenary to be presented at the 8th International Conference on Fetal Alcohol Spectrum Disorder: Research, Results, and Relevance, March 6-9th, 2019.
2. Petrenko CLM, Tapparello C, & Parr J (2019) Families Moving Forward – Connect: Developing a Mobile Health Intervention for Families Raising Children with Fetal Alcohol Spectrum Disorders. 90-min Symposia to be presented at the 8th International Conference on Fetal Alcohol Spectrum Disorder: Research, Results, and Relevance, March 6-9th, 2019.

We also had an abstract accepted as part of the CIFASD symposia for RSA in June, but that falls outside of the target window.

Petrenko/Tapparello U01 Intervention - Ns	Current Month	Cumulative Total	May 2019 Goal	% to Goal	Overall Goal	Completion Goal Date	May 2018 Goal	May 2019 Goal	May 2020 Goal	May 2021 Goal	May 2022 Goal
Focus Group Enrollment - YEARS 1 and 2											
Number of participants enrolled*	0	25	20	125.0%	20	2/28/2019	20	20	20	20	20
Number of groups conducted	0	7	6	116.7%	6	2/28/2019	6	6	6	6	6
Initial Feasibility Test - YEARS 1 and 2											
Number of participants enrolled	6	6	5	120.0%	5	5/31/2019	0	5	5	5	5
Number of interviews completed	0	0	5	0.0%	5	5/31/2019	0	5	5	5	5
*The number of and final sample size for focus groups will depend on data saturation in iterative thematic analysis.							Cumulative - at the end of each year.				

Petrenko/Tapparello U01 Intervention - Task %	% Complete	Completion Goal Date
FMF Connect App Development Major Milestones - YEARS 1 and 2		
Design interface programmed	90%	1/31/2018
Family Forum programmed	90%	5/31/2018
Psychoeducational content written & programmed	90%	7/31/2018
Audiovisuals produced & programmed	80%	9/30/2018
Initial Feasibility Prototype complete	85%	2/28/2019
	Start	**End**
Current month (defined by project) =	1/22/2019	2/25/2019
Date of project numbers update entry =	2/25/2019	

Families Moving Forward CONNECT

FMF Connect Components

Learning Modules
- 12 core modules, 3 levels
- Educational text/audio
- Exercises to practice content
- Animation and video

Notebook
- User builds personalized section for later reference
- Exercises about child, selected content, tools, notes

Dashboard
- Summary of progress
- Badges earned, child behavior ratings, usage metrics

Family Forum
- Users share ideas, ask questions, get support
- Organized in sub-forums
- Moderated by trained peers

Library
- Lists of books, websites, other resources
- Optional fact sheets

12/19/2018 CIFASD Reporting - Christie Petrenko & Cristiano Tapparello 1

Families Moving Forward CONNECT

Progress Update

- Learning Modules:
  - Programmed in Android, iOS in progress
  - Completed 3rd round of content edits to optimize for phone interface
  - Completed and reviewed ~55hrs of filming
    - 85% of rough cuts of video sequences completed
    - Selected voiceover artist
  - Initiated work with artist to design characters
- Family Forum
  - Most work completed in Yr. 1

12/19/2018 CIFASD Reporting - Christie Petrenko & Cristiano Tapparello 2

Progress Update (2)

- Library
  - 50% optional content written, remainder outlined and assigned
  - Will continue to grow during course of the project
- Notebook and Dashboard
  - Functionalities decided, mostly linkages with other components
- App-wide progress
  - Transitioned from Google Cloud Platform to Amazon Web Services for better integration and full HIPAA compliance
  - Overall tweaking of the user interface and functionalities
  - Planned manuscript describing the entire design and development process

Progress Update (3)

- Evaluation
  - 7 focus groups completed in 5 US cities
  - Transcribed verbatim, and line-by-line coded
  - Analysis near completion. Manuscript planned
  - Results to be presented in upcoming symposia

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Major Goals/Specific Aims

Risk for adult diseases or disorders is influenced by prenatal and early life environmental experiences. It is known that alcohol, in addition to its teratogenic effects, is an early life insult that programs developing systems and increases risk for diseases/disorders over the life course. Data from our animal model of prenatal alcohol exposure (PAE) suggest that fetal programming by PAE results in a sensitized, vulnerable organism with a pro-inflammatory bias that predisposes it to increased responsiveness to stress and immune challenges (second or third “hits”) over the life course, which in turn may underlie increased vulnerability to adverse health outcomes.

Our CIFASD Developmental Project (DP) is the first to identify links among maternal alcohol consumption, inflammation, and child outcomes; unique immune signatures in pregnant women were identified in association with both alcohol consumption and neurodevelopmental outcomes of their children. The proposed UO1 builds on these findings to examine immune profiles in pregnant women and children from birth to adulthood. This brings a significant new dimension to CIFASD, a focus on Developmental Origins of Health and Disease (DOHAD)/health outcomes, a critically important but relatively understudied area in the FASD field. Moreover, as the immune system plays a key role in brain development, aberrant immune/inflammatory mechanisms may also underlie FASD-associated neurobehavioral deficits that are well documented in the domains of neurocognition, self-regulation, and adaptive function. Our working hypothesis is that alcohol-induced dysregulation of immune/inflammatory function will be associated with adverse health, functional and adaptive outcomes, providing unique insight into factors underlying risk and resilience.

Aim 1. Use validation cohorts to confirm the utility of maternal and infant/child immune parameters as possible biomarkers and predictors of alcohol-related health and neurobehavioral outcomes. To determine the robustness of the immune signatures observed in our DP, we will: a) extend our collaboration with Dr. Chambers, using plasma samples from matched mother-infant pairs in her proposed new Ukraine cohort to further elucidate maternal influences on child outcome; b) extend analysis to samples from different cultural/ethnic, SES, and environmental conditions, to gain insight into factors modulating alcohol’s programming effects, through collaborations with: i) Drs. Chambers, Jones, Mattson: matched mother-child pairs from the San Diego FASD Research Subject Pool (Rady Children’s Hospital), and unexposed controls from other UCSD pediatric clinics; ii) Dr. Wozniak: new child cohort recruited for choline studies. Data from this research will increase our understanding of immune variables as biomarkers of alcohol intake and possible predictive factors for PAE-related health and neurobehavioral outcomes.

Aim 2. Extend our assessment of the immune system in individuals with FASD into adulthood. Since the studies of Streissguth and colleagues, few studies have investigated PAE effects into adult life, and of those that have, health issues have largely not been addressed. We will evaluate whether increased rates of physical/mental health problems and impairments in adaptive and functional outcomes in adults with FASD are associated with long-term immune system dysregulation resulting from prenatal programming effects of alcohol. Drs. Coles and Grant will recruit adults with: FAS, pFAS and alcohol effects; ARND; and unexposed controls in Atlanta and Seattle, respectively. In BC, with Drs. Loock, Oberlander, and Jan Lutke, we will recruit adults with: FAS, pFAS and alcohol effects; ARND; and unexposed controls. Blood samples will be analyzed for cytokines and other inflammatory markers. We will also evaluate past and current mental/physical health as well as functional and adaptive outcomes. These data will provide novel information on links among immune function, long-term adaptive and functional outcomes and health outcomes in adults with FASD.

Together, our proposed research will increase our understanding of immune variables as biomarkers of alcohol intake and predictive/possible mechanistic factors for PAE-related health and neurobehavioral outcomes.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Weinberg accomplishments.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded: WEINBERG TRAINING EXPERIENCES.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

i) REFEREED PAPERS:

Bodnar, T.S., Raineki, C., Wertelecki, W., Yevtushok, L., Plotka, L., Zymak-Zakutnya, N., Honerkamp-Smith, G., Wells, A., Rolland, M., Woodward, T.S., Coles, C.D., Kable, J.A., Chambers, C.D., Weinberg, J., CIFASD. (2018). Altered maternal immune networks are associated with adverse child neurodevelopment: Impact of alcohol consumption during pregnancy. Brain Behav Immun 73: 205-215.

Bodnar, T.S., Raineki, C., Wertelecki, W., Yevtushok, L., Plotka, L., Zymak-Zakutnya, N., Wells, A., Honerkamp-Smith, G., Coles, C.D., Kable, J.A., Chambers, C.D., J. Weinberg, the CIFASD. (in preparation). Immune disturbances following prenatal alcohol exposure: Implications for neurodevelopment.

ii) ABSTRACTS PUBLISHED:

Bodnar, T.S., Raineki, C., Wertelecki, W., Yevtushok, L., Plotka, L., Zymak-Zakutnya, N., Wells, A., Honerkamp-Smith, G., Coles, C.D., Kable, J.A., Chambers, C.D., J. Weinberg, the CIFASD. (2018). Cytokine disturbances associated with prenatal alcohol exposure in children: Implications for health and development. 41st Annual Scientific Meeting of the Research Society on Alcoholism. San Diego, CA, June 16-21. Alcohol Clin Exp Res, 42: 46A.

Bodnar, T., Raineki, C., Wertelecki, W., Yevtushok, L., Plotka, L., Zymak-Kakutnya, N., Wells, A., Honerkamp-Smith, G., Coles, C., Kable, J., Chambers, C., Weinberg, J. and the CIFASD (2018). Childhood cytokine profiles are altered by -prenatal alcohol exposure: Risk vs. resilience signatures. Dev Psychobiol 60 (Suppl 2):10.

Bodnar, T.S., Weinberg, J. and the CIFASD. Prenatal alcohol exposure disrupts the immune milieu: Impacts over the life course. In Symposium (Weinberg, Organizer and Chair): Neuroimmune dysfunction and health outcomes following prenatal alcohol exposure: Complementary cross-center perspectives. 42nd Annual Scientific Meeting of the Research Society on Alcoholism. Minneapolis, MN, June 22-26. Alcohol Clin Exp Res, in press

iii) PRESENTATIONS

Weinberg, J. (With: T. Bodnar, C. Raineki, W. Wertelecki, L. Yevtushok, N. Zymak-Zakutnya, A. Wells, G. Honerkamp-Smith, C.D. Coles, J.A. Kable, C.D. Chambers, and the CIFASD). Immune Dysregulation in FASD: Programming of Health and Neurobehavioral Outcomes. In: Plenary #1, CIFASD Symposium, PART 2 - Biomarkers of Exposure and Risk: The Quest for Early Diagnosis and Intervention. The 8th International Conference on FASD. Vancouver, BC, Canada. March 6-9, 2019.

Bodnar, T., Weinberg, J. Impact of prenatal alcohol exposure on immune function throughout the life course. CanFASD Sterling Clarren Award presentation. The 8th International Conference on FASD. Vancouver, BC, Canada. March 6-9, 2019.

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

There are no modifications or changes to the original plans.

During the next reporting period our plans include:

Aim 1. We will continue to work closely with our collaborators, Drs. Chambers, Jones, and Wozniak as they continue recruiting and testing participants for their studies. We will assist in organizing shipments of plasma samples to Vancouver as these studies proceed. Given the progress to date in these studies, we expect to start receiving shipments of plasma samples during the next reporting period. We will begin running assays to measure plasma levels of cytokines once sufficient numbers of samples are received. We need a minimum of 40 samples to run one plate, but we also need to balance across conditions within plates. As well, there are cost savings as well as increased replicability among plates if we place a larger rather than a smaller order for assay kits all from the same lot. These considerations will determine our assay schedule. As we obtain cytokine data, we will work with our collaborators in interpreting the data, and once we have sufficient data we will begin to write papers.

Aim 2. We will continue our regular meetings/conference calls with the Oberlander team and with Drs. Coles and Grant, respectively, to ensure that materials, protocols, and procedures remain consistent within and across sites. We will continue our active recruitment and testing of participants for the Adult Study. We will continue to extend our reach for recruitment through personal contacts, social media, and other means to increase our participant pool. We will work to refine our assay methodology for measurement of cytokine levels in blood spots prior to starting any assays on newborn blood spots from the San Diego study. As above, we will schedule cytokine assays according to the constraints noted, and will work with our colleagues in interpreting data and starting to write papers.

B.2. WEINBERG ACCOMPLISHMENTS

Aim 1. Use validation cohorts to confirm the utility of maternal and infant/child immune parameters as possible biomarkers and predictors of alcohol-related health and neurobehavioral outcomes. Our CIFASD Developmental Project (DP) is the first to identify links among maternal alcohol consumption, inflammation, and child outcomes: a) Analysis of blood samples from pregnant women identified unique immune signatures in association with both alcohol consumption and neurodevelopmental outcomes of their children; and b) Analysis of blood samples from children 2-3.5 years of age found unique immune signatures in the children depending on whether or not they had been exposed to alcohol and whether or not they show neurodevelopmental delay. Of note, although the pregnant women and children studies in this DP came from the same longitudinal cohorts, they were not matched.

Aim 1 of our UO1 builds on these findings and importantly, will assess *matched* mother-infant and mother-child pairs, to help *elucidate more fully maternal influences on child outcome*. With our collaborators, we will analyze blood samples from: A) matched mother-infant (Chambers), and B) mother-child (Jones, Chambers, Mattson) pairs; and C) Assess children 2-5 years of age from Dr. Wozniak's choline clinical trial. We will receive blood samples from our collaborators and analyze them for levels of cytokine, chemokines, angiogenesis factors and vascular injury markers. Health information is also being collected and will be sent to us. We will work with our collaborators to write papers based on the results of these studies. This Aim highlights the power of the CIFASD consortium in that we can leverage the activities and resources of CIFASD projects for our research.

To date, we have published one paper that provided the basis and direction for this UO1 (Bodnar et al., *Brain Behav Immun*, 2018). We utilized a multivariate approach to evaluate peripheral immune status, focusing on networks of interacting cytokines, chemokines, angiogenesis, and vascular markers in plasma, and investigated whether alterations in the maternal immune milieu could be linked to both alcohol-related and alcohol-independent neurodevelopmental delay in their children. This was based on the premise, supported by preliminary data, that dysregulation of the maternal immune system can result in deviations in the fetal cytokine balance, altering the course of typical brain development, and putting the individual on a "pathway to pathology". Plasma samples were obtained from pregnant women during the second and third trimesters of pregnancy, at two ONMI-Net sites in Western Ukraine, as part of the ongoing CIFASD longitudinal study in Ukraine (C. Chambers, PI). Importantly, we identified distinct clusters of activated/inhibited cytokines based on maternal alcohol consumption and child neurodevelopmental outcome. Specifically, cytokines, including IL-15, IL-10, MDC, and members of the VEGF sub-family, were highest in alcohol-consuming mothers of children with neurodevelopmental delay and were identified in both network analyses and examination of individual cytokines, whereas a differential and unique cytokine profile was identified in the case of alcohol-independent child neurodevelopmental delay. We propose that the current findings could provide a critical step towards the development of early biomarkers and possibly interventions for alcohol-related neurodevelopmental delay.

In addition, we have now completed a comprehensive examination of peripheral immune status in plasma samples from alcohol-exposed and unexposed children. Plasma samples were collected from children, 2–3.5 years old, born to women in the above-mentioned longitudinal study in Ukraine. Network analysis revealed differential patterns of cytokine expression depending on whether children were exposed to alcohol (A) *in utero* or were unexposed (C, controls), and on whether they showed neurodevelopmental delay (ND) or were typically developing (TD). As seen in Network 3, there were increases in eotaxin, eotaxin-3, and bFGF in alcohol-exposed children, independent of whether they showed neurodevelopmental delay or were typically developing, compared to unexposed control children. In addition, following alcohol-exposure, cytokine levels could be further differentiated based on child outcome. Specifically (Network 2), alcohol-exposed children with neurodevelopmental delay (A/ND) showed dampening of MIP-1β, MDC and MCP-4 expression, whereas these cytokines were activated in alcohol-exposed children who were typically developing (A/TD). Moreover, in children with neurodevelopmental delay, four cytokines (IL-2, TNF-β, IL-10, IL-15) showed dampened expression in alcohol-exposed, but increased expression in control children (Network 1), suggesting that these patterns

might be a marker of whether or not neurodevelopmental delay is due to alcohol. Of particular note, CRP levels were *elevated* in alcohol-exposed children with neurodevelopmental delay (A/ND) but *inhibited* in their neurotypical counterparts (A/TD) (Network 2). Together, these data suggest that changes in the peripheral immune balance during critical developmental periods may underlie some of the long-term effects of prenatal alcohol exposure on cognitive, physiological, and immune function, as well as the increased risk for mental health disorders later in life. A paper based on these results is almost completed and should be submitted within the next 2-3 months (Concept Proposal submitted).

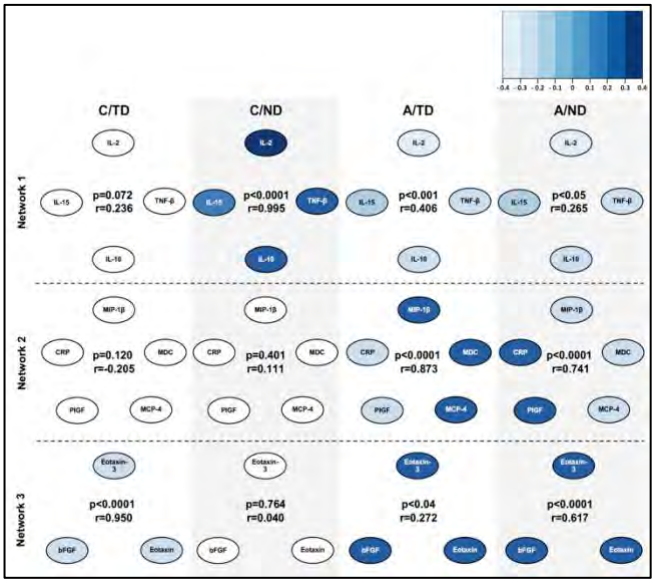


Fig. 1. Network analysis of child cytokine levels revealed 3 Networks, with differential patterns of cytokine inhibition (light blue) / activation (dark blue)

Aim 2. Extend our assessment of the immune system in individuals with FASD into adulthood. This study extends our research to the assessment of adults with FASD. We are collaborating closely with two groups: 1) Dr. Tim Oberlander and his team at British Columbia Children's Hospital who have extensive clinical and research experience with children with FASD as well as with adult subjects. In addition, Dr. Oberlander has a state-of-the-art Biobehavioral Testing Facility with well-appointed individual testing rooms, some with one-way mirrors from an observation room to a testing room, that we can use for testing our participants. The experience of the Oberlander team in testing both children and adults, and access to their testing facility are a great resource for us; and 2) Drs. Claire Coles (Emory University) and Therese Grant (University of Washington), who, in parallel with our study, will each recruit a cohort of adults with FASD and appropriate controls at their sites. We are collaborators on Dr. Coles' UO1 (Dr. Grant, Co-I) and she is a collaborator on our UO1. While there is some variation in the overall study design between our grants, the majority of the protocols and procedures overlap and will be consistent at the three sites. This will allow us not only to compare outcomes among the three sites, where participants come from diverse SES, ethnic, cultural and environmental backgrounds, but also to pool our data (with appropriate statistical control procedures) to increase our numbers significantly (pooled n=240 FASD/alcohol-exposed and 120 controls). This will increase the power of our study to: 1) correlate health status with individual cytokine profile, allowing a more nuanced interpretation of the data; and 2) link immune/health outcomes to behavior and adaptive functioning and investigate whether, in addition to its effects on physical and mental health, changes in the immune system are associated with the pervasive effects of prenatal exposure to alcohol on behavioral, cognitive, and adaptive function.

1) Major activities, June 2018 – May 2019

Aim 1.

A) Matched mother-infant pairs: We are extending our collaboration with Tina Chambers and team to obtain samples from her new Ukraine longitudinal cohort. Data are being collected on maternal demographics, characteristics, substance use, and health. Infant birth measures are also being collected. In addition, all infants will have a dysmorphology exam, as well as a 6 and 12 month Bayley. Recruitment in Ukraine is ongoing. To date, blood samples have been collected from 50 pregnant women, and deliveries are beginning. We are optimistic that we will be able to begin analyzing blood samples later this year.

B) Matched mother-child pairs: Recruitment and testing have now begun in San Diego. Children with FASD (~5-17 years) are being recruited from the San Diego FASD Research Subject Pool at Rady Children's Hospital-San Diego, consisting of a diverse set of children who have been evaluated for FASD by Drs. Ken Jones or Miguel del Campo. These children will have a dysmorphology exam,

complete a short health survey, and provide a blood sample. As well, for those born in San Diego County, we will have access to banked maternal mid-trimester plasma samples and child newborn blood spots. This is a potential gold mine of data – to have a longitudinal immune profile on the children and be able to match that to the maternal immune profile! We are optimistic that a substantial number of samples will be shipped to us later this year.

C) Dr. Wozniak's choline clinical trial is well underway. To date, 44 blood samples across 27 unique individuals have been collected. This provides a unique opportunity to investigate whether at least part of choline's beneficial effects may be due to reduced inflammation. We are hope to have a complete set of samples ready to analyze by spring or summer.

Aim 2.

By summer 2018, we completed all of our preparations for running the adult study: ethics applications approved; ethics training for UBC and NIH completed; Health Survey developed in partnership with Claire Coles and Therese Grant; protocols and forms for consent completed; Redcap license obtained, and all tests/instruments/ questionnaires received and entered into Redcap; Research Assistant hired and trained; training on hematology analyzer completed; cytokine panels finalized; protocols for taking 2-D pictures established; recruitment materials developed in consultation with Jan Lutke and Brenda Knight and distributed in the community; meeting held with Elders and Leaders of the First Nations community and support for our study confirmed (indeed, many are helping us with recruitment); Facebook page and website developed to advertise the study; advertisements placed on Craig's list; connections made with the Metis community and posters placed in the Longhouse, a UBC First Nations community center; recruitment outreach to justice system, Maria Keary Cottage, a transition house for offenders on conditional release or those who are homeless; all protocols, procedures, instruments pilot tested. As well, small shipments of blood samples were sent from both Atlanta and Seattle to ensure that logistics work when larger shipments are sent.

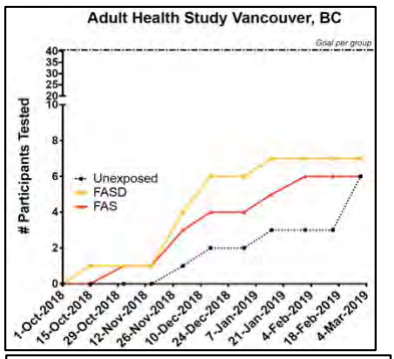


Fig. 2. Number of participants tested to date

We began contacting subjects to schedule appointments at the end of summer and formal testing of participants began in October 2018. To date (Fig. 2) we have tested 6 Unexposed Controls, 7 participants with FASD, and 6 participants with FAS. Further, 11 additional participants are scheduled between now and April 7th. Data from all individuals tested to date have been submitted for uploading to the CIFASD4 Central Repository, and 2D photos from 11 participants have been uploaded to the secure server (Suttie). Additional recruitment will again take place at the upcoming Vancouver FASD meeting. As well, Dr. Ken Jones has agreed to do dysmorphology exams on participants in our study while at the Vancouver meeting.

2) Specific Objectives:

Our Specific Objectives for the first few months of Year 2 were to complete all preparations and procedures and to initiate the studies for Aims 1 and 2. For Aim 1, we worked closely with our collaborators, Drs. Chambers, Jones, and Wozniak as they got their studies up and running to ensure that our collaborative goals were met. These collaborations epitomize the power of the CIFASD in enabling us to leverage resources to extend ongoing studies into novel domains. For Aim 2, we had regular (weekly for the first 5 months, now typically monthly unless issues arise) planning meetings/phone calls with the Oberlander team and with Drs. Coles and Grant, respectively, to put all materials, protocols, and procedures in place for the Adult Study.

For the remainder of Year 2, our objectives are to: a) Maintain regular contact with our Aim 1 collaborators; organize shipments of plasma samples to Vancouver as those studies proceed; and begin running assays for measurement of cytokines once sufficient numbers of samples have been received; and b) Continue active recruitment and testing of participants in our Adult Study; continue outreach through personal contacts, social media, and other means to increase our participant pool; refine our

assay methodology for measurement of cytokine levels in blood spots; and begin running assays to measure cytokines once sufficient numbers of samples have been collected.

3) Significant Results:

Analysis of cytokines, chemokines, angiogenesis, and vascular markers in plasma of pregnant women identified distinct clusters of activated/inhibited cytokines associated with both maternal alcohol consumption and child neurodevelopmental outcome. Specifically, cytokines, including IL-15, IL-10, MDC, and members of the VEGF sub-family, were highest in alcohol-consuming mothers of children with neurodevelopmental delay and were identified in both network analyses and examination of individual cytokines, whereas a differential and unique cytokine profile was identified in the case of alcohol-independent child neurodevelopmental delay. In addition, examination of peripheral immune status in alcohol-exposed and unexposed children revealed differential immune profiles in alcohol-exposed compared to unexposed children, that were also linked to whether or not they showed neurodevelopmental delay. We propose that the current findings could provide a critical step towards the development of early biomarkers and possibly interventions for alcohol-related neurodevelopmental delay.

4) Key Outcomes and Other Achievements:

Studies for Aims 1 and 2 are ongoing. While we do not yet have either sufficient numbers of participants tested nor sufficient numbers of plasma samples collected from any of these studies, we strongly believe that the novel and exciting findings from our preliminary studies provide a strong basis for the promise of exciting and novel findings in the present studies.

TRAINING EXPERIENCES

We welcome *undergraduate students* to join our laboratory and participate in research, supervised by graduate students, Postdoctoral Fellows and Research Associates, with oversight by Dr. Weinberg. These students, together with laboratory members participate in monthly laboratory meetings and a laboratory journal club covering important research papers relevant to the field.

Work-learn students (Stipends from the University to support undergraduate students working in research laboratories). Srishti Sarkar and Nirmal Seerha have been Work-learn students in my laboratory for the past year. They have contributed actively to ongoing laboratory research projects, including our CIFASD project.

The *Research Assistant* for our CIFASD project, Amanda Chao (formerly a Work-learn student in my laboratory), has been trained extensively to become proficient in all procedures and activities related to our study and is now taking a lead role in recruiting, scheduling and running the Study Day. In addition, she has completed the NMED 1117, Basic Venipuncture for Allied Health Professionals, at the BC Institute of Technology, Vancouver, BC, and along with Dr. Tamara Bodnar, a Co-I on this study, is taking blood samples from all of our subjects.

Specific training activities related to undergraduate students in the laboratory include the following:

- **UNDERGRADUATE TRAINING**
  - Directed Readings
    - Monthly lab journal club covering influential research relevant to the field
  - Psychology 366 research project:
    - Srishti Sarkar - Winter 2018
  - Summer Student Research Program (Scholarship)
    - Cecilia Fung
  - UBC Undergraduate Neuroscience Conference
    - Srishti Sarkar: Sarkar, S., Bodnar, T.S., Rainki, C., Weinberg, J. Effects of prenatal alcohol exposure and early-life adversity on immune function. UBC Undergraduate Neuroscience Conference, Vancouver, BC, January 31st, 2019.
    - Nirmal Seerha: Seehra, N., Bodnar, T., Sarkar, S., Raineki, C., Weinberg, J. The effects of prenatal alcohol exposure and early-life adversity on neuroimmunity. UBC Undergraduate Neuroscience Conference, Vancouver, BC, January 31st, 2019.
  - Multidisciplinary Undergraduate Research Conference 2018
    - Srishti Sarkar: Sarkar, S., Bodnar, T.S., Holman, P., Weinberg, J. Effects of minocycline administration of spatial learning and memory performance following prenatal alcohol exposure. Multidisciplinary Undergraduate Research Conference, Vancouver, BC, March 16, 2018.

- Work learn students:
    - Srishti Sarkar
    - Nirmal Seerha

❖ OUTREACH ACTIVITIES

- Expert Advisory Committee for 8th International Research Conference on Adolescents and Adults with Fetal Alcohol Spectrum Disorder (2017-18) & 8th International Conference on Fetal Alcohol Spectrum Disorder (2018-19)
    - Student Representative - Parker Holman
- Fetal Alcohol Spectrum Disorders Study Group (FASDSG) (2018 - 2019)
    - Post-doc Representative - Tamara Bodnar
- UBC Faculty of Medicine Center for Excellence in Indigenous Health:
    - Health Sciences Pre-admissions Workshop
        - Samantha Baglot - Volunteer

❖ SYMPOSIA, RETREATS & MEETINGS

- Attendance at weekly Neuroscience Research Colloquia
- Attendance at monthly Neuroscience "Pizza Seminar"
- Attendance at weekly Cellular and Physiological Sciences Departmental Seminars
- Attendance at the monthly microbiome seminar series and journal club
    - Tamara
    - Samantha
- Attendance at weekly Behavioral Neuroscience Seminar (Dept. of Psychology)
    - Parker Holman
    - Charlis Raineki
- Conference attendance
    - 8th International Research Conference on Adolescents and Adults with FASD, Vancouver, BC, April 18-21, 2018
        - Parker Holman
        - Charlis Raineki
        - Tamara Bodnar
        - Samantha Baglot
        - Amanda Chao
    - 41st Annual Research Society on Alcoholism Conference. San Diego, CA, June 16-20, 2018.
        - Parker Holman
        - Charlis Raineki
        - Tamara Bodnar
        - Samantha Baglot

- 48th Annual Meeting of the Society for Neuroscience, San Diego, CA, Nov. 3-7, 2018.
  - Charlis Raineki
  - Tamara Bodnar
  - Samantha Baglot
- 51st Annual Meeting of the International Society for Developmental Psychobiology. San Diego, CA, Oct. 31-Nov. 2
  - Charlis Raineki
  - Tamara Bodnar
  - Samantha Baglot
- 19th Congress of International Society for Biomedical Research on Alcoholism ISBRA 2018. Kyoto, Japan. Sept 9-13, 2018.
  - Tamara Bodnar
- Neuroscience Extravaganza Poster, December 5, 2018
  - Charlis Raineki
  - Parker Holman

❖ **AWARDS & HONORS**

- Charlis Raineki
  - Best poster in the "Postdoctoral Fellow/Research Associate – Behavioral" category at the 2018 UBC Neuroscience Extravaganza
- Tamara Bodnar:
  - Travel Award - NIH / Sackler Travel Award for ISDP
  - Mitacs Accelerate Internship
  - CanFASD Sterling Clarren FASD Research Award
  - CIHR Indigenous Gender and Wellness Travel Award
- Samantha Baglot:
  - Travel Award - NIH / Sackler Travel Award for ISDP
  - Lindsay & Elizabeth Gordon Award in Health Sciences (UBC) - $5,000
  - Harold F. and Anne Bedner Uphill Scholarship in Health Sciences (UBC) - $5,000

Describe your project's interrelation with aims of the CIFASD consortium its other projects and the progress on the interactions.

1) We are extending our collaboration with Dr. Tina Chambers and team to obtain samples from matched mother-infant pairs being recruited in her new Ukraine longitudinal cohort. Data are being collected on maternal demographics, characteristics, substance use, and health. Infant birth measures are also being collected. In addition, all infants will have a dysmorphology exam, as well as a 6 and 12 month Bayley. Recruitment in Ukraine is ongoing. To date, blood samples have been collected from 50 pregnant women, and deliveries are beginning. We are optimistic that we will be able to begin analyzing blood samples later this year.

2) We are collaborating with Drs. Jones, Chambers and Mattson on a study of children. Children with FASD (~5-17 years) are being recruited from the San Diego FASD Research Subject Pool at Rady Children's Hospital-San Diego, consisting of a diverse set of children who have been evaluated for FASD by Drs. Ken Jones or Miguel del Campo. These children will have a dysmorphology exam, complete a short health survey developed by us, and provide a blood sample. As well, for those born in San Diego County, we will have access to banked maternal mid-gestation plasma samples and child newborn blood spots. Control (unexposed) children will be recruited in collaboration with Mattson (as per Aim 1 of her UO1) or through other studies currently underway in Dr. Chambers' laboratory, and plasma samples and health questionnaire data obtained through Jones and Chambers. Physical examination and blood sampling on all alcohol-exposed and unexposed children will be done by Dr. Jones. Recruitment and testing have now begun, and we are optimistic that a substantial number of samples will be shipped to us later this year.

3) We are collaborating with Dr. Wozniak on his choline clinical trial. He will provide us with plasma samples from children with FASD, receiving choline or placebo (this collaboration not part of Wozniak's CIFASD UO1). Dr. Wozniak's choline clinical trial is well underway. To date, 44 blood samples across 27 unique individuals have been collected. This provides a unique opportunity to investigate whether at least part of choline's beneficial effects may be due to reduced inflammation. We are hope to have a complete set of samples ready to analyze by spring or summer.

4) We are extending our assessment of the immune system in individuals with FASD into adulthood, in close collaboration with Dr. Claire Coles (Dr. Grant, University of Washington, Co-I); in parallel, we are each (Atlanta, Seattle, Vancouver) recruiting cohorts of adults with FASD and appropriate controls. While there is some small variation among sites, the majority of our protocols and procedures overlap and will be consistent at the three sites. This will allow us not only to compare outcomes among the three sites, where participants come from diverse SES, ethnic, cultural and environmental backgrounds, but also to pool our data (with appropriate statistical control procedures) to increase our numbers significantly (pooled n=240 FASD/alcohol-exposed and 120 controls). Recruitment is well underway at all three sites. Small shipments of plasma samples have already been received from Atlanta and Seattle to ensure that logistics will work for larger sample shipments. We have regular conference calls to plan, trouble shoot, and ensure that we are consistent among the three sites.

5) Weinberg is providing 2D facial pictures of adults with FASD and controls to Dr. Suttie for his analysis. We have communicated regularly with Dr. Suttie on the protocol for taking photos and have already uploaded photos from participants to date to his secure site.

6) Aliquots of plasma from blood samples taken from infants in Dr. Chambers' new Ukraine birth cohort will be analyzed by Weinberg (cytokines), Miranda (miRNA), and Hashimoto-Torii (epigenetic marks). With three of us analyzing samples from the same infants, we can interact to determine whether the use of multiple biomarkers can provide more sensitive indicators of

risk/resilience than any single biomarker alone. We will all share data and work together to investigate this possibility. Once blood samples have been collected and sent, the four of us will connect to begin to discuss analyses and sharing of data.

7) Dr. Weinberg will be interacting with Drs. Mooney and Blanchard to share data over the course of their studies. Animal model studies of Mooney and Blanchard build on data from our animal model work demonstrating alterations in immune/inflammatory function following prenatal alcohol exposure. Moreover, Preliminary Data from our Developmental Project, demonstrating that immune signatures in pregnant women consuming/not consuming alcohol are associated with child outcome will be considered by Drs. Mooney and Blanchard in the interpretation of his microbiome data from maternal females and offspring. Finally, new data to be collected in our proposed UO1 will be considered in Dr. Blanchard's project using both qRT-PCR and ELISA analysis of stimulated spleen cells from alcohol-exposed and control rat pups. Thus, our data on pregnant women and their infants/children and or adults exposed/unexposed to alcohol can be validated in Dr. Blanchard's animal model; conversely, microbiome data from Dr. Blanchard's animal model can be considered in the interpretation of our data on cytokine profiles, health and neurobehavioral outcomes from our clinical samples. As above, we have not yet interacted, but will interact extensively once samples have been collected and analyzed.

8) We are collecting saliva samples on all of our participants for Drs. Foroud and Weatherill to include in their genetic analyses. In addition, at the upcoming Vancouver FASD Conference, we are sharing a table in the Exhibit area with Drs. Foroud/Weatherill to recruit participants for both of our studies, and sharing a small room where Drs. Foroud/Weatherill can collect saliva from volunteers, and where Dr. Ken Jones can perform dysmorphology exams on participants recruited for our adult study.

Inclusion Enrollment Report

Inclusion Data Record (IDR) #: 173963

Using an Existing Dataset or Resource: No

Delayed Onset Study ?: No

Clinical Trial: No

Enrollment Location: Foreign

NIH Defined Phase III Clinical Trial: No

Study Title: Immune Dysregulation in FASD: Programming of Health and Neurobehavioral Outcomes

Planned Enrollment

Comments: More than One Race = Aboriginal (to be recruited in British Columbia, Canada)

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/Alaska Native	0	0		0	0					**0**
Asian	0	0		0	0					**0**
Native Hawaiian or Other Pacific Islander	0	0		0	0					**0**
Black or African American	0	0		0	0					**0**
White	30	30		0	0					**60**
More than One Race	30	30		0	0					**60**
Unknown or Not Reported										
Total	**60**	**60**		**0**	**0**					**120**

Cumulative Enrollment

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/Alaska Native	0	0	0	0	0	0	0	0	0	**0**
Asian	0	0	0	0	0	0	0	0	0	**0**
Native Hawaiian or Other Pacific Islander	0	0	0	0	0	0	0	0	0	**0**
Black or African American	0	0	0	0	0	0	0	0	0	**0**
White	0	0	0	0	0	0	0	0	0	**0**
More than One Race	0	0	0	0	0	0	0	0	0	**0**
Unknown or Not Reported	0	0	0	0	0	0	0	0	0	**0**
Total	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	Petrelli B, Weinberg J, Hicks GG. Effects of prenatal alcohol exposure (PAE): insights into FASD using mouse models of PAE. Biochemistry and cell biology = Biochimie et biologie cellulaire. 2018 April;96(2):131-147. PubMed PMID: 29370535; PubMed Central PMCID: PMC5991836.
Complete	Bodnar TS, Raineki C, Wertelecki W, Yevtushok L, Plotka L, Zymak-Zakutnya N, Honerkamp-Smith G, Wells A, Rolland M, Woodward TS, Coles CD, Kable JA, Chambers CD, Weinberg J. Altered maternal immune networks are associated with adverse child neurodevelopment: Impact of alcohol consumption during pregnancy. Brain, behavior, and immunity. 2018 October;73:205-215. PubMed PMID: 29738852; PubMed Central PMCID: PMC6344127.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Category	Explanation
Research Material	www.weinberglab.ca. Our laboratory website describes: ongoing research projects andactivities; laboratory personnel, including undergraduates, graduate students, Postdoctoral Fellows, and Research Associates, and their activities, awards, honors and accomplishments; and current news and findings of interest to the FASD field as a whole.

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period? No

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization?

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

WEINBERG CIFASD INTERNAL PROGRESS REPORT

*Publications [Accepted & In Press]

Bodnar, T.S., Raineki, C., Wertelecki, W., Yevtushok, L., Plotka, L., Zymak-Zakutnya, N., Honerkamp-Smith, G., Wells, A., Rolland, M., Woodward, T.S., Coles, C.D., Kable, J.A., Chambers, C.D., Weinberg, J., CIFASD. (2018). Altered maternal immune networks are associated with adverse child neurodevelopment: Impact of alcohol consumption during pregnancy. *Brain Behav Immun* 73: 205-215.

*Publications [In Preparation & Submitted]

Bodnar, TS, Raineki, C, Wertelecki, W, Yevtushok, L, Plotka, L, Zymak-Zakutnya, N, Honerkamp-Smith, G, Wells, A, Coles, CD, Kable, JA, Chambers, CD, Weinberg, J, and the CIFASD. Immune disturbances following prenatal alcohol exposure: Implications for neurodevelopment. In preparation (Concept Proposal submitted February 23, 2019).

* Poster Abstracts and Presentations

i) Abstracts Published:

Bodnar, T.S., Raineki, C., Wertelecki, W., Yevtushok, L., Plotka, L., Zymak-Zakutnya, N., Wells, A., Honerkamp-Smith, G., Coles, C.D., Kable, J.A., Chambers, C.D., J. Weinberg, the CIFASD. (2018). Cytokine disturbances associated with prenatal alcohol exposure in children: Implications for health and development. 41st Annual Scientific Meeting of the Research Society on Alcoholism. San Diego, CA, June 16-21. *Alcohol Clin Exp Res*, 42: 46A.

Bodnar, T., Raineki, C., Wertelecki, W., Yevtushok, L., Plotka, L., Zymak-Kakutnya, N., Wells, A., Honerkamp-Smith, G., Coles, C., Kable, J., Chambers, C., Weinberg, J. and the CIFASD (2018). Childhood cytokine profiles are altered by -prenatal alcohol exposure: Risk vs. resilience signatures. Dev Psychobiol 60 (Suppl 2):10.

Bodnar, T.S., Weinberg, J. and the CIFASD. Prenatal alcohol exposure disrupts the immune milieu: Impacts over the life course. In Symposium (Weinberg, Organizer and Chair): Neuroimmune dysfunction and health outcomes following prenatal alcohol exposure: Complementary cross-center perspectives. 42nd Annual Scientific Meeting of the Research Society on Alcoholism. Minneapolis, MN, June 22-26. *Alcohol Clin Exp Res*, in press.

ii) Presentations

Weinberg, J. (With: T. Bodnar, C. Raineki, W. Wertelecki, L. Yevtushok, N. Zymak-Zakutnya, A. Wells, G. Honerkamp-Smith, C.D. Coles, J.A. Kable, C.D. Chambers, and the CIFASD). Immune Dysregulation in FASD: Programming of Health and Neurobehavioral Outcomes. In: Plenary #1, CIFASD Symposium, PART 2 - Biomarkers of Exposure and Risk: The Quest for Early Diagnosis and Intervention. The 8th International Conference on FASD. Vancouver, BC, Canada. March 6-9, 2019.

Bodnar, T., Weinberg, J. Impact of prenatal alcohol exposure on immune function throughout the life course. CanFASD Sterling Clarren Award. The 8th International Conference on FASD. Vancouver, BC, Canada. March 6-9, 2019.

Weinberg U01 Immune Dysregulation - YEARS 2 & 3	Current Month	Cumulative Total	May 2019 Goal	% to Goal	Overall Goal	Completion Goal Date	May 2018 Goal	May 2019 Goal	May 2020 Goal	May 2021 Goal	May 2022 Goal
Cytokine Samples Received - Mother-Infant Pairs and Children Plasma Samples											
Ukraine - Chambers											
PG Mother AE	0	0	20	0.0%	30	5/31/2020	0	20	30	30	30
PG Mother CON	0	0	20	0.0%	30	5/31/2020	0	20	30	30	30
Infant at birth AE	0	0	20	0.0%	30	5/31/2020	0	20	30	30	30
Infant at birth CON	0	0	20	0.0%	30	5/31/2020	0	20	30	30	30
San Diego - Chambers and Jones											
Biobank 3rd-tri mother and infant at birth AE	0	0	15	0.0%	40	5/31/2021	0	15	30	40	40
Biobank 3rd-tri mother and infant at birth CON	0	0	15	0.0%	40	5/31/2021	0	15	30	40	40
Child (age 5-17) AE	0	0	15	0.0%	40	5/31/2021	0	15	30	40	40
Child (age 5-17) CON (Mattson identified?)	0	0	15	0.0%	40	5/31/2021	0	15	30	40	40
Minnesota - Wozniak											
Child (age 2.5-5) AE Placebo	0	0	15	0.0%	30	5/31/2020	0	15	30	30	30
Child (age 2.5-5) AE Choline	0	0	15	0.0%	30	5/31/2020	0	15	30	30	30
Cytokine Samples Received - Adult Samples											
Vancouver Adults (Weinberg and Oberlander)											
Adults FASD	1	5	15	33.3%	40	5/31/2020	0	15	30	40	40
Adults AE	1	7	15	46.7%	40	5/31/2020	0	15	30	40	40
Adults CON	2	5	15	33.3%	40	5/31/2020	0	15	30	40	40
Atlanta Adults (Coles)											
Adults FASD	0	3	15	20.0%	40	5/31/2021	0	15	30	40	40
Adults AE	0	4	15	26.7%	40	5/31/2021	0	15	30	40	40
Adults CON	0	1	15	6.7%	40	5/31/2021	0	15	30	40	40
Seattle Adults (Grant)											
Adults FASD	0	1	15	6.7%	40	5/31/2021	0	15	30	40	40
Adults AE	0	4	15	26.7%	40	5/31/2021	0	15	30	40	40
Adults CON	0	2	15	13.3%	40	5/31/2021	0	15	30	40	40
Adult Testing											
Vancouver Adults (Weinberg and Oberlander)											
Tested FASD	1	5	15	33.3%	40	5/31/2020	0	15	30	40	40
Tested AE	1	7	15	46.7%	40	5/31/2020	0	15	30	40	40
Tested CON	2	5	15	33.3%	40	5/31/2020	0	15	30	40	40
Adult Recruitment											
Vancouver Adults (Weinberg and Oberlander)											
Adults recruited	15	85	100	85.0%	120	5/31/2020	50	100	120	120	120

	Start	End
Current month (defined by project) =	1/21/2019	2/20/2019
Date of project numbers update entry =	2/20/2019	

Cumulative - at the end of each year.

WEINBERG UO1: SPECIFIC AIM 1:

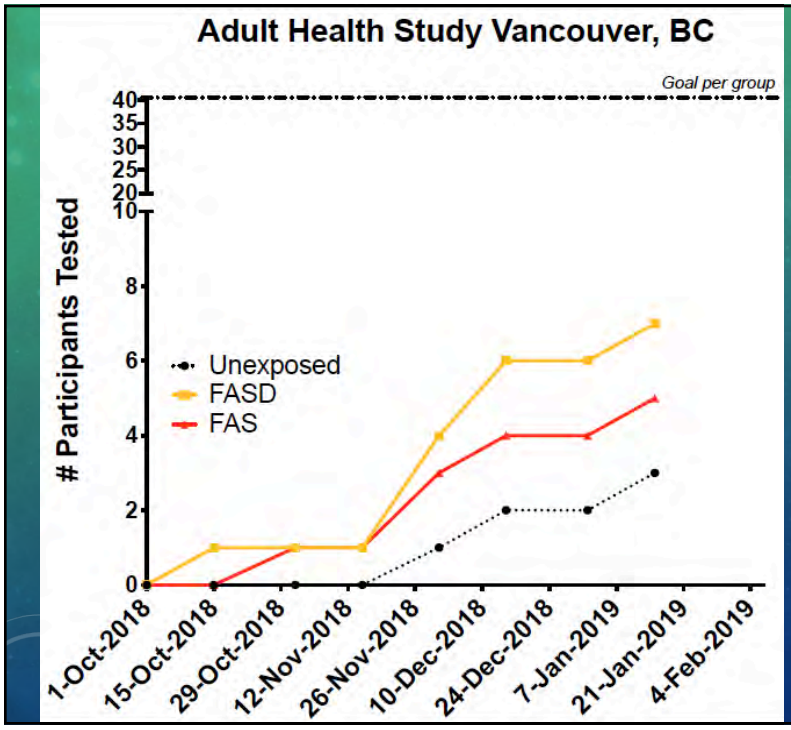
Use validation cohorts to confirm the utility of immune parameters as possible biomarkers and predictors of alcohol-related health and neurobehavioral outcomes:

- With Chambers: Extend collaboration to new Ukraine cohort - *matched mother-infant pairs* - RECRUITMENT ONGOING – BLOOD SAMPLES FROM 50 PREGNANT WOMEN, DELIVERIES BEGINNING (1 INFANT SAMPLE TO DATE)
- With Jones, Chambers, Mattson: Children ~5-17 yr (Jones' San Diego Registry) – SAMPLE COLLECTION UNDERWAY
- With Wozniak – children (2-5 yr), new choline clinical trial – 44 SAMPLES ACROSS 27 UNIQUE INDIVIDUALS COLLECTED TO DATE

WEINBERG UO1: SPECIFIC AIM 2

Extend assessment of the immune system into adulthood with assessment of adults, ~22-50+ years, recruited in British Columbia [and in close collaboration with UO1 of Coles (Atlanta) (and with Grant, Seattle)]

- RECRUITMENT AND TESTING NOW ONGOING
  - REGULAR CONFERENCE CALLS with Claire and Therese to coordinate methods, testing, blood sampling, etc.
  - SMALL SHIPMENTS OF BLOOD SAMPLES FROM SEATTLE AND ATLANTA to confirm that the logistics will work
  - FACEBOOK PAGE developed for recruitment
  - Like Claire, reach out to justice system: MARIA KEARY COTTAGE – Transition house for offenders on conditional release or those who are homeless
  - LONGHOUSE at UBC
  - METIS COMMUNITY
  - Recruitment again at FASD 2019 VANCOUVER CONFERENCE



ADULT STUDY, VANCOUVER, BC

Testing began in October
Progress to date:

- 3 Controls
- 7 FASD
- 5 FAS

- Data from 12 participants submitted for for uploading to the CIFASD4 Central Repository
- 2D photos from 11 participants uploaded to server (Suttie)

B. ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Aim 1: Perform a comprehensive characterization of the FASD “connectome” using state-of-the-art Human Connectome Project (HCP) methods in children with PAE (combining functional and structural imaging).

Hypothesis 1a: The full range of FASD will show abnormalities relative to controls in one or more domains: network efficiency, cortical gyrification, white matter integrity, & intra-cortical myelin. In addition to a control group, the HCP Development project (a study of typical development in 1300 children) will allow us to evaluate these abnormalities against a full psychometric characterization of typical development in non-exposed children.

Hypothesis 1b: Subtle abnormalities in cortical development and connectivity will identify a sizeable proportion of children impacted by PAE who do not meet full criteria for FASD and would be otherwise undiagnosable.

Aim 2: Evaluate the sensitivity and specificity of commonly-applied diagnostic criteria (alcohol exposure, dysmorphology, growth, and cognition) to identify individual children with underlying neurodevelopmental abnormalities. This will provide objective evaluation of various diagnostic criteria for FASD (which remain contentious).
Hypothesis 2: Alcohol exposure variables, growth characteristics, and dysmorphic features will each have unique sensitivity, specificity, & positive/negative predictive value for identifying neurodevelopmental abnormalities.

Aim 3. Fully characterize relationships between connectomics and cognitive functioning.

Hypothesis 3: Connectivity abnormalities will be associated with deficits in cognitive functioning – especially low IQ, poor attention, slow processing speed, and impaired executive functioning.

Aim 4: Examine the developmental course of connectivity, gyrification, and myelin in children with FASD. Participants will undergo longitudinal re-assessment at 15-months (matched to the HCP-development paradigm (HCP-D)). This will allow for comparison of change over time to a large longitudinal dataset being collected as part of the HCP-D project.

Hypothesis 4: Longitudinal analyses will demonstrate abnormal development over time in PAE compared to controls. Typical development will be associated with greater changes in cortical development, myelin status, and connectivity/network efficiency with age compared to PAE - which will be associated with a more static, impaired neurodevelopmental course.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Progress_Report Accomplishments Wozniak 2-2019.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required?

No

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

During the next budget period, we plan to continue enrollment in order to stay on track toward the targets. We are on track as of today and projections indicate that we will remain on track this coming budget period. In March, 2019, we have scheduled the first of our 15-month follow-up visits with participants. All participants will return for a second MRI scan and shortened neurocognitive evaluation to allow for planned longitudinal analyses. The coming year will bring analyses of both cognitive data and brain imaging data, including some analyses that are currently underway.We will continue to collect ancillary samples and data for our collaborators including plasma for Dr. Joanne Weinberg's immune function project, plasma for Dr. Miranda's micro-RNA project, 3D images for Dr. Michael Suttie's project, and cognitive data for Dr. Sarah Mattson's project. We are also assisting Dr. Christie Petrenko with recruitment of families who have children with FASD for focus groups to assist in the development of her smartphone application.

Principal Investigator(s): Jeffrey R. Wozniak, Ph.D.
Institution(s): University of Minnesota
CIFASD4 Project Title: Multi-modal connectivity methods for the validation of Fetal Alcohol Spectrum Disorder diagnostic criteria
Grant Number: 1U01AA026102

B.2 What was accomplished under these goals?

1) Major Activities.

- Total participants enrolled or currently scheduled as of 2/19/2019 is 58 (target is 60 by 5/31/18)
- Participants with completed MRIs, 3D facial images, and neurocognitive testing as of 2/19/2019 is 50.
- Within the last year, we hired a new MR technician for scanning and imaging data processing.
- Structural MRI data have been processed and segmented volumetric data have been prepared for sharing with other CIFASD investigators (and eventually external investigators) via Indiana University's Central Repository (Indiana is preparing to receive the data via direct upload)
- Acquisition of 3D facial images for Dr. Michael Suttie's project: 50 as of 2/19/2019
- Blood samples obtained from Dr. Wozniak's R01 project (R01AA024123-01) to provide to collaborator Dr. Joanne Weinberg's CIFASD project; Collected thus far = 50 samples across 30 unique participants.
- Dr. Kenneth Jones visited the University of Minnesota in October and conducted dysmorphology exams on 36 children and adolescents enrolled in CIFSD. All of these participants also provided MRI and neurocognitive data.
- Salivary DNA (n=36) and 2D photographs (n=50) were collected from CIFASD participants and shared with Dr. Tatiana Foroud and Leah Wetherill at Indiana University. All of these participants also provided dysmorphology data, MRI, and neurocognitive data
- Neurocognitive and behavioral data were collected and shared in a joint database with Dr. Sarah Mattson at SDSU for her Decision Tree diagnostics project.

2) Specific Objectives.

- Year 2 (6/1/2018 – 5/31/2019) total target = 33.

3) Significant Results.

Data collection is underway, so significant results are not yet available from this project. However, we are actively analyzing data from both the previous phase of CIFASD (CIFASD-III) and publishing as well as, now, actively starting to analyze (and share) CIFASD-IV data.

4) Key Outcomes and Other Achievements.

Currently on target or ahead of schedule for recruitment.

Describe your project's interrelation with aims of the CIFASD consortium its other projects and the progress on the interactions.

- This project is most closely tied with Dr. Sarah Mattson's project. We are collecting the same neurocognitive data at both sites and sharing data. In addition, we are assisting Dr. Mattson in testing the Decision Tree software. To date, we have collected these data for 50 participants.
- We continue to collect 3D facial images to be shared with Dr. Michael Suttie's project. To date, we have collected these data from 50 participants.
- We will continue to collect blood from Dr. Wozniak's R01 study on choline intervention to be shared with Dr. Joanne Weinberg as part of her CIFASD-IV project. Collected thus far = 50 samples across 30 unique participants.
- This project interfaces closely with the Dysmorphology project. Dr. Kenneth Jones visited the University of Minnesota in October and conducted dysmorphology exams on 36 children and adolescents enrolled in CIFSD. All of these participants also provided MRI and neurocognitive data.
- This project is also interfacing with the Indiana University project on genetics and facial imaging. Salivary DNA (n=36) and 2D photographs (n=50) were collected from CIFASD participants and shared with Dr. Tatiana Foroud and Leah Wetherill at Indiana University. All of these participants also provided dysmorphology data, MRI, and neurocognitive data
- This project has also interfaced with Dr. Christie Petrenko's project. We have initiated a connection with our local state organization (Proof Alliance) to facilitate recruitment for Dr. Petrenko's project and we have been distributing recruitment materials to our research participants as well.

Inclusion Enrollment Report

Inclusion Data Record (IDR) #: 173964

Using an Existing Dataset or Resource: No

Delayed Onset Study ?: No

Clinical Trial: No

Enrollment Location: Domestic

NIH Defined Phase III Clinical Trial: No

Study Title: Multi-modal connectivity methods for the validation of Fetal Alcohol Spectrum Disorder diagnostic criteria

Planned Enrollment

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/Alaska Native	6	6		0	0					**12**
Asian	1	1		0	0					**2**
Native Hawaiian or Other Pacific Islander	0	0		0	0					**0**
Black or African American	5	5		0	0					**10**
White	23	23		6	6					**58**
More than One Race	3	3		1	1					**8**
Unknown or Not Reported										
Total	**38**	**38**		**7**	**7**					**90**

Cumulative Enrollment

Comments: Includes completed and scheduled but not yet enrolled

Racial Categories	Ethnic Categories									Total
	Not Hispanic or Latino			Hispanic or Latino			Unknown/Not Reported Ethnicity			
	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	Female	Male	Unknown/ Not Reported	
American Indian/Alaska Native	0	1	0	0	0	0	0	0	0	**1**
Asian	1	0	0	0	0	0	0	0	0	**1**
Native Hawaiian or Other Pacific Islander	0	0	0	0	0	0	0	0	0	**0**
Black or African American	3	1	0	0	0	0	0	0	0	**4**
White	3	7	0	0	0	0	0	0	0	**10**
More than One Race	2	0	0	0	0	0	0	0	0	**2**
Unknown or Not Reported	0	1	0	0	0	0	0	0	0	**1**
Total	**9**	**10**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**19**

C. PRODUCTS

C.1 PUBLICATIONS

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph) during the reporting period resulting directly from this award?

Yes

Publications Reported for this Reporting Period

Public Access Compliance	Citation
Complete	Suttie M, Wozniak JR, Parnell SE, Wetherill L, Mattson SN, Sowell ER, Kan E, Riley EP, Jones KL, Coles C, Foroud T, Hammond P. Combined Face-Brain Morphology and Associated Neurocognitive Correlates in Fetal Alcohol Spectrum Disorders. Alcoholism, clinical and experimental research. 2018 September;42(9):1769-1782. PubMed PMID: 29935097; PubMed Central PMCID: PMC6120799.

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Category	Explanation
Audio or video	Wozniak, J.R. (2/22/2018). “Fetal Alcohol Spectrum Disorders”, Minnesota Public Radio (MPR) News on air interview and call-in show with host Mike Mulcahy; https://www.mprnews.org/story/2018/02/22/fetal-alcohol-disorders-may-be-more-common
Audio or video	Wozniak J.R. (7/23 & 7/24/2018). “Fetal alcohol disorders are more common than you think”, televised story for PBS NewsHour and accompanying web piece by Amna Nawaz; (Over 99,000 views of the video story on Facebook); https://www.pbs.org/newshour/show/fetal-alcohol-disorder-is-more-common-than-you-think
Audio or video	Wozniak J.R. (7/24/2018). “The story about drinking while pregnant that got our newsroom talking”, web piece for PBS NewsHour by Amna Nawaz, Lorna Baldwin, and Dr. Amber Robbins; https://www.pbs.org/newshour/nation/the-story-about-drinking-while-pregnant-that-got-our-newsroom-talking
Audio or video	Wozniak, J.R. & Rossman, J. (1/20/2019). “Fetal Alcohol Spectrum Disorders”, News and Views radio show on WCCO Radio with Roshini Rajkumar. https://wccoradio.radio.com/media/audio-channel/1-20-19-news-views-roshini-rajkumar-1-pm

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Have inventions, patent applications and/or licenses resulted from the award during the reporting period?

If yes, has this information been previously provided to the PHS or to the official responsible for patent matters at the grantee organization?

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

Principal Investigator(s): Jeffrey R. Wozniak, Ph.D.
Institution(s): University of Minnesota
CIFASD4 Project Title: Multi-modal connectivity methods for the validation of Fetal Alcohol Spectrum Disorder diagnostic criteria
Grant Number: 1U01AA026102

Publications [Accepted & In Press]

Doyle, L.R., Moore, E.M., Coles, C.C., Kable, J.A., Sowell, E.R., Wozniak, J.R., Jones, K.L., Riley, E.P., Mattson, S.N., and the CIFASD (2018). Executive Functioning Correlates with Communication Ability in Youth with Histories of Heavy Prenatal Alcohol Exposure. Journal of the International Neuropsychological Society, 24(10): 1026-1037. PMID: 300322415; PMCID: PMC6237635;

Suttie, M.M., Wozniak, J.R., Wetherill, L.M., Mattson, S.M., Sowell, E.R., Kan, E., Riley, E.P., Jones, K.L., Coles, C.C., Foroud, T., Hammond, P., & the CIFASD (2018). Combined face-brain morphology and associated neurocognitive correlates in Fetal Alcohol Spectrum Disorders. Alcoholism: *Clinical and Experimental Research.* PMID: 29935097; PMCID: PMC6120799

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

Wozniak, J.R., Riley, E.P., & Charness, M.E. (commissioned, 3rd revision under review). The lifelong impact of prenatal alcohol exposure on brain development and function. Lancet Neurology. Impact Factor: 26.28; Citations: 0; Contributions: Literature review, manuscript preparation.

***Poster Abstracts and Presentations**

Wozniak, J.R. (2018). FASD Research Update: Brain Imaging and the Development of Smart Interventions, keynote address at the 7th Annual FASD Matters Conference, Brooklyn Park, Minnesota.

Wozniak U01 Neuroimaging	Current Month	Cumulative Total	May 2019 Goal	% to Goal	Overall Goal	Completion Goal Date	May 2018 Goal	May 2019 Goal	May 2020 Goal	May 2021 Goal	May 2022 Goal
MRI Scan #1											
MRI Scan #1 - PAE	1	33	30	110%	45	5/31/2020	15	30	45	45	45
MRI Scan #1 - CON	0	23	30	77%	45	5/31/2020	15	30	45	45	45
Cognitive Evaluation (Mattson NB Battery)											
Cognitive evaluation - PAE	1	33	30	110%	45	5/31/2020	15	30	45	45	45
Cognitive evaluation - CON	0	23	30	77%	45	5/31/2020	15	30	45	45	45
MRI Scan #2											
MRI Scan #2 - PAE	YR3	YR3	0	YR3	30	1/1/2022	0	0	10	20	30
MRI Scan #2 - CON	YR3	YR3	0	YR3	30	1/1/2022	0	0	10	20	30

	Start	End
Current month (defined by project) =	1/15/2019	2/21/2019
Date of project numbers update entry =	2/21/2019	

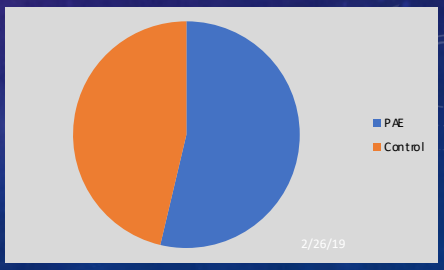
Cumulative - at the end of each year.

NEUROIMAGING PROJECT UPDATE

Jeff Wozniak, University of Minnesota, 1-23-2019

Progress:

- 56 Enrolled and completed first MRI scan and cognitive evaluation)
- May 31 goal = 60 (93% of target; well on-track)
- 56 / 90 = 62% of final target
- Longitudinal: Second scans (15 months) will begin March, 2019



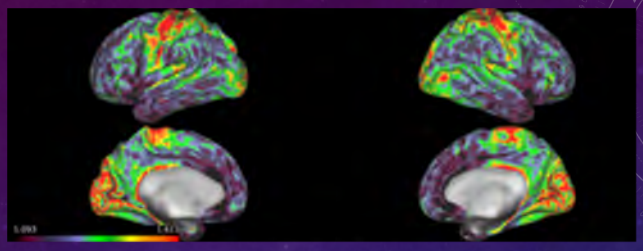


Figure 4. Intra-cortical myelin map of a control participant.

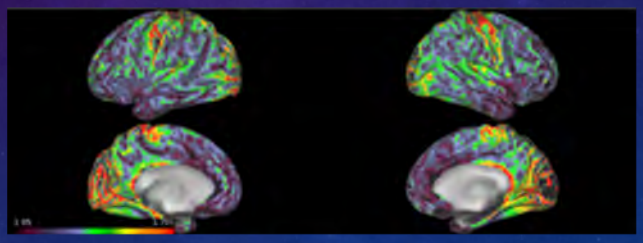
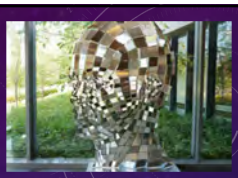


Figure 5. Intra-cortical myelin map of a participant with PAE.

INTERACTIONS



BLOOD SAMPLES (Weinberg project)

- Collecting blood for choline treatment study (pre and post)
  - Saving plasma for Dr. Weinberg
- N=44 collected thus far (27 unique individuals)

FACE (Suttie project)

- Collecting 3D faces (new handheld camera)
  - CIFASD + another study (N=53+20=73)

DYSMORPHOLOGY (Jones project)

- N=37 participants seen

NEUROBEHAVIOR (Mattson project)

- All participants for imaging have "validation" neuropsych battery
- N=56 (53 uploaded to "tree" app)

2/26/19

Principal Investigator(s): Thomas Blanchard, Ph.D., Sandra Mooney, Ph.D.
Institution(s): University of Maryland School of Medicine; University of North Carolina Nutrition Research Institute
CIFASD4 Project Title: Prenatal alcohol effects on the gut microbiome contributing to failure to thrive and altered immune function.
Grant Number: 5UH2AA026109-02

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

Aim 1. Characterize the changes in the gut microbiome associated with alcohol consumption in pregnant rat dams and the acquisition of the altered microbiome by nursing pups.
Aim 2. Define changes in the nature of the immune response of pups born and nursed by alcohol-fed dams.
Aim 3. Determine the potential for correcting the alcohol-associated gut microbiome to normal in pups born to alcohol-fed pregnant dams.

B.1.a Have the major goals changed since the initial competing award or previous report? The major goals have not changed.

B.2 What was accomplished under these goals?

1) Major Activities. Preliminary studies were performed using qRT-PCR to evaluate a gross measure of fecal microbiota composition in control and ethanol-exposed adult female rats. Specific taxa included in that analysis were Bacteroidetes, Firmicutes, Gammaproteobacteria, and a universal primer that identifies all microbiota (total bacterial load). Gross analysis indicated that exposure of the rats to ethanol during pregnancy was not sufficient to alter the microbial composition of the gut flora as determined from fecal samples. To address this issue an alternative strategy was adopted in which non-pregnant females were purchased and given long term exposure to alcohol consumption administered either in liquid diet or in the drinking water. Rats were treated for a minimum of 6 to 8 weeks. qRT-PCR did not reveal changes in the marker bacteria. However, experiments were continued based on the likelihood that qRT-PCR lacked the power to identify changes and that a microarray on the entire microbiome would be necessary. Females on chronic alcohol consumption were mated and treatment was continued throughout pregnancy. Dams and pups were harvested 30 days post delivery for sample collection. All samples (fecal pellet DNA, serum, spleen cell culture supernatants, and small and large bowel tissue) are frozen and awaiting analysis.

2) Specific Objectives. The specific objectives are to 1) determine if alcohol consumption by adult female rats induces dysbiosis of the gut microbiota, 2) if this altered gut flora is adopted by their offspring, 3) if in utero exposure of pups to alcohol changes the nature of immune system development, and 4) if an altered gut flora in pups born to alcohol fed dams influences the nature of the immune response. Although the appropriate alcohol exposures and sample collections were performed, addressing each of these objectives is reliant upon the analysis of the collected samples. This analysis will be performed in the coming months.

3) Significant Results. Two modified in utero alcohol exposure strategies were employed to optimize the likelihood of inducing dysbiosis of the gut flora in pregnant rats. Fecal pellet DNA, serum, spleen cell culture supernatants from stimulated cells, and small and large bowel tissue have been collected from all pups at harvest. No results can be reported until these samples are used for microbiome analysis and multiplex cytokine array analysis. These activities will be accomplished in the final few months of the grant period.

It is of note that the breeding of these rats was more challenging than anticipated. Our goal was to achieve litters from 5 groups of pregnant females, those on an ethanol-based liquid diet, those on a control liquid diet, those receiving ethanol in their drinking water, and those given control water. Although we have attempting these breedings for the better part of a year we were able to achieve

pregnancy with 4 females receiving the ethanol liquid diet and none from the control diet. We also achieved pregnancies with 3 females given ethanol in water and 4 liters from those with control water. This was in spite of repeated and prolonged breeding attempts and the purchase of additional animals.

4) Key Outcomes and Other Achievements. It is impossible to state the key outcomes from these experiments as analysis for microbiome populations and cytokine profiles has not been performed yet.

B.4 What opportunities for training and professional development has the project provided? Nothing to report

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

B.6 Modified - What do you plan to do during the rest your project's funding period to accomplish the goals set forth by your UH2?

We have collected fecal samples from pups exposed to alcohol in utero from dams given a liquid diet containing ethanol, or normal chow with water containing ethanol. We have also collected samples from the dams before and after ethanol treatment. Total DNA has been isolated from all samples. These DNA samples will be analyzed for 16S RNA Gene sequences at the University of Maryland Institute for Genomic Sciences to determine and compare the microbiome composition of each group. Alcohol exposed pups will be compared to non-alcohol exposed control animals as well as against the profiles of their corresponding dams post-alcohol treatment. These data will determine a) the extent to which long-term alcohol consumption alters the gut flora of adult, pregnant females, and b) if the gut flora of pups exposed to alcohol in utero resembles that of the dam, that of control pups, or a mix of characters.

Blood samples have also been collected from the dams and pups at harvest and the serum stored at -80^{o}C. These samples will be tested for a panel of 27 cytokines in a rat cytokine array using a multiplex array. Cytokine protein levels will be determined and the averages compared between groups. These data will determine to what extent alcohol exposure changes the character of immune homeostasis based on cytokine profiles.

In addition to blood samples, the spleens for each pup were used to isolate spleen cells for in vitro pan Tcell stimulation. The T cells were stimulated by a combination of anti-CD34 and anti-CD28. Spleen cells were recovered after 24 hours of stimulation and the supernatants were recovered and frozen at -80^{o}C. These samples will be tested using the 27 cytokine multiplex array to determine if exposure to alcohol in utero changes the character of the immune response compared to control pups.

Approximately 10 cm of distal small bowel and descending colon were collected at harvest, washed and frozen at -80^{o}C. If a change in immune profiles is noted by cytokine multiplex array these tissues will be assessed by qPCR for the cytokines identified in the multiplex assay to change the most between groups. They will also be used for immunohistochemistry of frozen sections to measure the prevalence of T cell subsets.

***Publications [Accepted & In Press]** None
***Publications [In Preparation & Submitted]** None
***Poster Abstracts and Presentations** None

Describe your project's interrelation with aims of the CIFASD consortium its other projects and the progress on the interactions.

Our project has the most potential to interact studies on the immune system of pregnant females in which it was demonstrated that a signature cytokine profile was present during the second trimester in women drinking during pregnancy. Analysis of the cytokine array of blood collected from the dams may demonstrate consistencies.

Our study on the microbiota of gut of dams and pups may provide a strong impetus for testing of similar samples from the human cohort followed in Europe to determine if the children of mothers that drank during pregnancy have a signature bacterial profile that either negatively influences the character of the immune system or could serve as marker for exposure to alcohol in utero.

Blanchard Mooney UH2 Microbiome		**Date of Update:**	2/21/2019	Table to keep is in the loop on the poop. :)			
Aim / Experiment	**# proposed**	**Status May 2018**	**Current Month**	**Cumulative Total**	**Overall Goal**	**% Complete**	**Projected Status May 2019**
Aim 1 changes in gut microbiome							
Experiment 1.1 dam	10 dams - ET in water (5 control 5 ethanol-exposed), see note 1	20 rats in house, 5 per group	0	7	10	70%	completed
	10 dams - ET in diet (5 control 5 ethanol-exposed), see note 2		0	4	10	40%	completed
Experiment 1.2 pup	40 offspring (1 M, 1 F per litter), see note 3	0	0	22	40	55%	completed
Aim 2 immune response in pups							
Experiment 2 in vitro	40 offspring (1 M, 1 F per litter)	0	0	22	40	55%	completed
Aim 3 correction of microbiome							
Experiment 3 fecal microbiota transfer	20 offspring (1 M, 1 F per ethanol-treated litter)	0	0	0	20	0%	completed

Note 1: 4 control & 3 ethanol

Note 2: 4 ethanol, no controls

Note 3: tissues collected from 2 males and 2 females per litter

Samples Collected from Experimental Animals

- Dams:
  - Stool samples collected every two weeks - microbiome
  - Blood collected after weaning litter and before euthanizing – cytokine profiling
- Offspring :
  - Stool collected at P21(at weaning) and P50 - microbiome
  - Spleen at P60 - cytokine profiling
  - Blood at P60 - cytokine profiling
  - Intestinal tissue at P60 – histology & identification of specific cell types
  - Whole brain at P60 – banked for later analysis

Status of Alcohol Microbiome 12/14/2018

	Liquid Diet Control	ETOH Liquid Diet	Water Treatment Control	Water Treatment ETOH
# of litters receiving treatment	5	5	5	5
# of litters collected from as of 12/14/2018	0	3	4	3

Principal Investigator(s): Kazue Hashimoto-Torii, Masaaki Torii
Institution(s): Children's National
CIFASD4 Project Title: Biomarker for intellectual disability in children prenatally exposed to alcohol
Grant Number: 5UH2AA026106-02

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

Aim1: Establish novel peripheral biomarkers for predicting the risk of cognitive and learning deficits in mice prenatally exposed to ethanol.

Aim2: Assess the peripheral biomarkers in human FASD subjects.

As the subcontract institute, the Chambers lab will collect human blood samples, perform neurocognitive tests, and statistically evaluate potential correlations between the test scores and RNA profiles. This project will allow critical assessment of the link between biomarkers and comprehensive measures of neurocognitive deficits, brain structural abnormalities and facial dysmorphology. In addition, these studies will maximize the value of our collaborations with other CIFASD research projects including the neurobehavioral (Chambers), genetic (Foroud) and dysmorphology core (Jones) projects. The cross-sectional approach using controlled animal studies (Eberhart and Parnell) will provide additional mechanistic insights. Biomarkers identified from our study and those obtained through the studies using cytokine (Chambers) and miRNA (Weinberg) panels generated for the same PAE patients will provide a rare opportunity to test the combined biomarker strategy for accurate prediction of PAE outcomes. By capitalizing on CIFASD infrastructure, this project will develop innovative biomarkers that impact the FASD research.

B.1.a Have the major goals changed since the initial competing award or previous report? No

B.2 What was accomplished under these goals?

1) Major Activities. We have completed RNA sequencing of T cell, B cell, and monocytes, and neurobehavior assessment in animal models in Aim1. In Aim 2, we established human pipeline of analysis, and successfully collected two patient samples sent from UCSD Chambers' cohort.

2) Specific Objectives. Using the RNA sequencing data, we defined the mRNA biomarkers using multiple bioinformatics tools.

3) Significant Results. We found ~ 20 genes as biomarkers that can separate the bad and good learners in FASD animals with the accuracy of ~0.89 using Random Forest.

4) Key Outcomes and Other Achievements. We are currently wrapping up animal data for manuscript.

B.3 Competitive Revisions/Administrative Supplements

For this reporting period, is there one or more Revision/Supplement associated with this award for which reporting is required? Yes; UH2AA026106-02S1

The goal of the admin supplement is to increase the number of human samples collected in Aim 2. This is based on Aim 1 animal studies that found the low power of decision making with the originally proposed sample number.

B.4 What opportunities for training and professional development has the project provided? Nothing to report

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

B.6 Modified - What do you plan to do during the rest your project's funding period to accomplish the goals set forth by your UH2? Continue to collect human blood samples, and perform RNA sequencing. Write manuscripts for animal studies.

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

Are there publications or manuscripts accepted for publication in a journal or other publication (e.g., book, one-time publication, monograph, or preprint) during the reporting period resulting directly from this award? Nothing to Report

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

Mohammad S., et al., Reduction of excessive Kcnn2 ameliorates learning disability in the mouse model of Fetal Alcohol Spectrum Disorders. Manuscript in revision in Nature Neuroscience, submission form had been submitted to CIFASD in 2018 spring.

***Poster Abstracts and Presentations**

Sasaki J, Sasaki T, Banerjee P, Igawa A, Lazarski C, Niba V, Carryl H, Mohammad S, Imamura Kawasawa Y, Torii M, Hashimoto-Torii K. Peripheral RNA biomarkers for the prediction of neurocognitive deficits in the mouse models of FASD and gestational diabetes. 9th International Research Conference on Adolescents and Adults with FASD, March 2019

Mohammad, S., Hashimoto-Torii, K. CELL-TO-CELL VARIABLE MOLECULAR RESPONSES FOR MAINTENANCE OF PROTEOSTASIS. Symposium @ RSA, June 2019

Torii, M. Genome-wide Profiling of altered RNA splicing in human fetal cortical tissue exposed to alcohol. Symposium @ RSA, June 2019

Describe your project's interrelation with aims of the CIFASD consortium its other projects and the progress on the interactions.

We have been receiving patient blood samples that are shared between Chambers, Miranda and Weinberg. The patients have been evaluated by Jones and Mattson. Correlation of our RNA seq data and the behavior scores will be examined. From blood samples, we banked the white blood cells that will be potential resource of genomics studies that are performed by such as Eberhart, Parnell and Foroud groups. Plasma is also collected and stored for potential usage such as immune response profiling (Weinberg) and miRNA profiling (Miranda, Chambers).

Torii U01	Collections / Analysis / Tests Completed					
Aim 1 Mice	**Goal Samples**	**T Cell collection**	**B Cell collection**	**Sequencing**	**Data analysis**	**Behavior test**
Collected samples (PBS)	10	30	30	75	60	60
Collected samples (EtOH)	20	30	30	75	60	60
Aim 2 Ukraine Human Infants & School-age	**Goal Samples**	**T Cell collection**	**B Cell collection**	**Sequencing**	**Data analysis**	
Collected samples (no exposure)	10	0	0	0	0	
Collected samples (exposure)	30	2	2	0	0	
Date of Update:	2/21/2019					

Peripheral RNA Biomarkers for Intellectual Disability in FASD

Aim1: Mouse Biomarkers

Wet part (behavior test, RNA seq) had been completed.

Testing several machine learning system;
1. Random Forest (~0.9 with ~10 top dispersed genes X ~60 dataset)
2. Deep learning with Keras on Tensorflow (in process)

Aim2: Human Biomarkers

The analysis pipeline had been established.

From first 1.6ml FASD blood , following samples were successfully collected.
White blood cell RNA (T cell, B cell, monocytes)
Plasma (stored)
T cell, B cell, monocytes (stored for genomics study)