CIFASD4 February 2018 Face-to-Face Meeting Internal Progress Reports		
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Principal Investigator: Edward P. Riley
Institution: San Diego State University
CIFASD4 Project Title: Administrative Core of the CIFASD
Grant Number: U24AA014811

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.

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. 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 to achieve the aims of all consortium components.

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

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. The CIFASD4 Notice of Grant Awards were received the first part of July 2017 and it consists of two cores (U24s), including the AdminC, nine U01 research projects and two UH2 developmental projects. Organizationally, the AdminC is led by an Advisory Committee composed of Dr. Edward Riley, PI of the AdminC and the Consortium Coordinator, Dr. Michael Charness, the Scientific Director of CIFASD, Dr. Jennifer Thomas, the Administrative Specialist, the Science Advisory Board (Drs. John Hannigan, Sara Jo Nixon and Dan Savage), and the NIAAA advisors (Drs. Bill Dunty, Dale Hereld). Since the current notice of award, the AdminC has coordinated and moderated the monthly teleconference meetings of the CIFASD4 PIs and Advisory Committee members. The July meeting was an introductory meeting and guest speaker Sylvia Roozen, an FASD researcher from The Netherlands, presented on *"FASD prevention and the way forward"*. During the August meeting, each project presented an outline of its particular interactions with other CIFASD4 projects. Beginning with the September meeting, project PIs have presented their individual project's progress to the group via WebEx presentations: Dr. Sarah Mattson in September, Dr. Peter

Hammond in October, Dr. Jeff Wozniak in November and Drs. Claire Coles, Christie Petrenko and Cristiano Tapparello in December. During the October meeting, guest speaker Olivia Weeks, a PhD candidate at Harvard Medical School, presented her work involving prenatal alcohol exposure and metabolism in zebrafish. Through the invitation of guest speakers, Drs. Riley and Charness introduce external investigators to CIFASD as well as provide consortium members with new perspectives and access to outside experts. Updates on the status of CIFASD publications are prepared by the AdminC and shared with the group during each monthly meeting.

An orientation and training for new CIFASD4 investigators on the CIFASD publications policy and its accompanying forms was provided by the AdminC in September 2017. Dr. Thomas is an ex officio member of the Data Access Committee and the Publication Policy Committee; each committee has held meetings during this reporting period. Drs. Thomas and Riley participate in their conference call meetings and email communication strings. Both are also in regular communication with the Informatics team to facilitate access to CIFASD data from previous phases to external investigators. Dr. Thomas also ensures the CIFASD.org website is up-to-date. During this reporting period, the Research tab was updated to showcase the newly funded CIFASD4 projects. Archives of the mp3 recordings and shared slide sets from the CIFASD4 monthly meetings in the secure section of the website are also maintained by the AdminC.

Recently, the AdminC has been organizing and coordinating the first face-to-face annual meeting of CIFASD4. The meeting will take place February 5-6, 2018 in Rockville, MD. Progress reports are being collected from all CIFASD4 projects for review by the Advisory Committee prior to the meeting. At the meeting, each project will also present slides to update all consortium members on their progress. Guest experts are also being invited to participate and present at the meeting. Currently planned are presentations on the Children's Brain Health Network, data sharing strategies, and eHealth. Finally, the Advisory Committee will have a closed meeting to discuss the progress of CIFASD4.

In addition to facilitating the dissemination of CIFASD's scientific findings through NOFAS, results are also presented at scientific conferences. Dr. Charness assembled a symposium submitted for consideration for the RSA meeting to be held in San Diego, CA in June 2018, entitled, "Predicting Outcomes of Fetal Alcohol Exposure in the CIFASD Cohort." The introduction would be given by Dr. Charness and presentations from CIFASD investigators would include: Dr. Scott Parnell on transient ciliopathy, Mike Suttie on 3D facial analysis, Dr. Joanne Weinberg on immune function, and Dr. Christina Chambers on growth modeling. Drs. Charness and Riley would serve as discussants. Dr. Charness is also organizing a symposium of CIFASD science for consideration at the next ISBRA Congress to be held in Kyoto, Japan in September 2018.

Working with NIAAA advisors, Drs. Bill Dunty and Dale Hereld, the AdminC has been assisting with the creation and coordination of the CIFASD4 progress tracking documents. All CIFASD projects have drafted tables to illustrate their projects' main aims with the focus on data and sample collection. Discussion of how to finalize the tables will take place at the face-to-face meeting in February 2018. The end goal is to have clean and clear visualizations of the progress of each project.

During the final year of Phase III of CIFASD, Dr. Riley recruited Dr. Ganapathy "Ganz" Chockalingam, a computer scientist and engineer, to assist CIFASD with its growing interest in developing and utilizing mobile apps and online eHealth technologies. With the start of CIFASD4, Dr. Chockalingam now operates through a subaward from the AdminC and he and Dr. Riley meet regularly. Ganz has continued his work with Dr. Sarah Mattson, a CIFASD4 U01 PI, to adapt her decision tree for screening individuals with FASD to a tablet platform and integrate it with data collected in other projects. Dr. Chockalingam has assisted with the design and development of the first prototypes of the decision tree tablet (iPad) app and web portal that will automate Dr. Mattson's decision tree algorithm. The goal is to utilize the functionality of the tablet to enroll subjects at various locations, input behavioral questionnaire responses directly, store neurobehavioral scores, capture dysmorphology measures and use the automated decision tree algorithm to render a risk score. Considerable progress has been made; however, further refinements are necessary to iron out bugs and to add new features before the prototype is implemented. Dr. Chockalingam has also been communicating with Drs. Christie Petrenko and Cristiano Tapparello regarding their CIFASD4 U01 project and providing consultation on their

development of an app framework for interventional strategies. Ganz will be attending the face-to-face meeting in February 2018 to interface with other PIs to determine how he can assist their projects.

The AdminC maintained the allocation of shared resources during this reporting period. A portable Canfield Vectra 3D handheld Canon camera and laptop were delivered to Dr. Jeff Wozniak at the University of Minnesota to resolve a space storage issue UMN was having with an older 3D camera model. The Bellus3D face camera tool and tablet continue their residence with Mike Suttie at the University of Oxford.

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?

Both Drs. Riley and Charness serve on the Expert Planning Committee for the 8th International Research Conference on Adolescents and Adults with FASD to be held in Vancouver, BC, Canada in April 2018. The international FASD meetings held in Vancouver provide excellent platforms for caregivers, health care professionals, social workers, educators, policymakers, scientists and individuals with FASD to interact and learn from one another.

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

Dr. Riley has been working with NIAAA to secure funding to continue the dissemination of CIFASD research findings and outreach efforts provided by NOFAS in the previous iteration of this consortium. The CIFASD website also provides information not only about individual projects and their accomplishments, but also about FASD in general. In October 2017, Dr. Riley gave a plenary lecture at the 16th Congress of the European Society for Biomedical Research on Alcoholism (ESBRA) in Crete, Greece highlighting recent CIFASD findings. Finally, Drs. Charness and Riley continue to organize symposium sessions of CIFASD findings for presentation at national and international scientific conferences.

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

The AdminC will conduct evaluations to ensure that all CIFASD4 projects are progressing at a suitable level. Additionally, the AdminC will continue to monitor the status of shared samples and supply chains to assist with keeping all projects on target with their timelines and goals. The AdminC is also looking to expand the current three-member Science Advisory Board to include one or more new members.

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

NIH Public Access Compliance	Citation
Complete	Infante MA, Moore EM, Bischoff-Grethe A, Tapert SF, Mattson SN, Riley EP. Altered functional connectivity during spatial working memory in children with heavy prenatal alcohol exposure. Alcohol. 2017 Nov;64:11-21. doi: 10.1016/j.alcohol.2017.05.002. Epub 2017 Aug 12. PubMed PMID: 28965651; PubMed Central PMCID: PMC5635832.
Complete	Kable JA, Coles CD; CIFASD.. Prefrontal cortical responses in children with prenatal alcohol-related neurodevelopmental impairment: A functional near-infrared spectroscopy study. Clin Neurophysiol. 2017 Nov;128(11):2099-2109. doi: 10.1016/j.clinph.2017.08.009. Epub 2017 Sep 2. PubMed PMID: 28914230; PubMed Central PMCID: PMC5675790.
Complete	Wozniak JR, Mueller BA, Mattson SN, Coles CD, Kable JA, Jones KL, Boys CJ, Lim KO, Riley EP, Sowell ER; CIFASD.. Functional connectivity abnormalities and associated cognitive deficits in fetal alcohol Spectrum disorders (FASD). Brain Imaging Behav. 2017 Oct;11(5):1432-1445. doi: 10.1007/s11682-016-9624-4. PubMed PMID: 27734306; PubMed Central PMCID: PMC5389933.
Complete	Taggart TC, Simmons RW, Thomas JD, Riley EP. Children with Heavy Prenatal Alcohol Exposure Exhibit Atypical Gait Characteristics. Alcohol Clin Exp Res. 2017 Sep;41(9):1648-1655. doi: 10.1111/acer.13450. Epub 2017 Aug 21. PubMed PMID: 28727159; PubMed Central PMCID: PMC5581268.
Complete	Suttie M, Wetherill L, Jacobson SW, Jacobson JL, Hoyme HE, Sowell ER, Coles C, Wozniak JR, Riley EP, Jones KL, Foroud T, Hammond P; CIFASD.. Facial Curvature Detects and Explicates Ethnic Differences in Effects of Prenatal Alcohol Exposure. Alcohol Clin Exp Res. 2017 Aug;41(8):1471-1483. doi: 10.1111/acer.13429. Epub 2017 Jul 10. PubMed PMID: 28608920; PubMed Central PMCID: PMC5563255.
Complete	Gross LA, Moore EM, Wozniak JR, Coles CD, Kable JA, Sowell ER, Jones KL, Riley EP, Mattson SN; CIFASD.. Neural correlates of verbal memory in youth with heavy prenatal alcohol exposure. Brain Imaging Behav. 2017 Jun 27. doi: 10.1007/s11682-017-9739-2. [Epub ahead of print] PubMed PMID: 28656347; PubMed Central PMCID: PMC5745322. [Epub ahead of print]

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

Drs. Charness, Riley and Wozniak are preparing an article for *The Lancet Neurology*.

***Poster Abstracts and Presentations**

"Fetal Alcohol Spectrum Disorders (FASD). An overview," plenary lecture delivered by Dr. Edward Riley at the 16th Congress of the European Society for Biomedical Research on Alcoholism (ESBRA), Crete, Greece, October 2017.

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

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

If a project you were the PI on was part of Phase III of CIFASD, use this space to update the Advisory Committee on any new progress not previously reported.

A formal carryover request was submitted in mid-January 2018 to request additional time to use unliquidated obligated funds to Dr. Kirsty Donald's (University of Cape Town) and Dr. Joanne Weinberg's (University of British Columbia) developmental projects from Phase III. CIFASD4 marks a change for developmental projects as they are now being awarded as separate UH2 grants and therefore, report on their progress is not required by the AdminC. However, if funds are made available to developmental projects from Phase III through the carryover mechanism, updates on their progress will be included within the AdminC's progress report.

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? No.

B.2 What was accomplished under these goals?

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 06/01/2017 and 01/23/2018, using the CIFASD physical examination protocol and classification system, Dr. Jones performed nine infant/children physical examinations in San Diego, CA. In addition, using the CIFASD examination training Dr. Jones has worked face-to-face and trained six health care providers (five in Ukraine and one in San Diego, CA).

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. Nine completed physical examinations at the San Diego, CA, site and six health care providers trained.

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

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 FASDs, 2) train additional physicians in the clinical identification of FASDs and 3) recruit additional research subjects who have been prenatally exposed to alcohol

1) Major Activities. Dr. Jones has applied for a Medical Staff appointment at the Zuni Indian Health Center in Zuni, NM, and is awaiting approval.

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. None. We are still waiting for the Medical Staff appointment to be approved.

4) Key Outcomes or Other Achievements. Medical Staff appointment application was submitted and is pending approval.

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 06/01/2017 and 01/23/2018, through the FASD Clinic at RCHSD, we have enrolled 34 new subjects (22 males and 12 females) into the FASD Research Subject Pool bringing our total registry numbers up to 127. We have referred 35 subjects from this research pool to Dr. Sarah Mattson's Decision Tree project. Additionally, to support Dr. Joanne Weinberg's project, we are in the process of obtaining 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. We anticipate to receive IRB approval in the next month.

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 34 new subjects (22 males and 12 females) into the FASD Research Subject Pool bringing our total registry numbers up to 127. In addition, we have referred 35 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 are awaiting approval.

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 from June 1, 2017- January 23, 2018.

Category	N
Number of in person CIFASD completed dysmorphology examinations by location	Atlanta, GA = 0 Minneapolis, MN = 0 San Diego, CA = 9 Seattle, WA = 0 Ukraine = 0 Vancouver, BC = 0
Number of physicians trained using the CIFASD examination training protocol	Total = 6 (5 Ukraine and 1 San Diego)
Number subjects recruited into the FASD research registry	Male = 22 Female =12 Total for the reporting period = 34 Total in registry = 127
Telemedicine	Minnesota & San Diego (convergent validity of telemedicine **Yrs. 1-2**): Children with FAS = 0 Children without FAS = 0 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 = Children without FAS = San Diego (training telemedicine **Yrs. 2-4**): Pediatric residents trained = 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 Mattson U01 (**Yrs. 1-5**) Number of referrals made = 35 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.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?

During this reporting period, Dr. Jones has trained health care providers in Ukraine and in San Diego, CA. He has also been trained Pediatricians, Geneticists and Neonatologists who attend his diagnostic 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 92 people.

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

Results from this project period have been disseminated to the communities of interest through three publications.

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 to conduct dysmorphology exams and begin the telemedicine project in Western New Mexico at the Indian Health Hospital and in Minnesota. In addition, we plan to start 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 complete an age appropriate questionnaire.

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

NIH Public Access Compliance	Citation
Complete	Wozniak JR, Mueller BA, Mattson SN, Coles CD, Kable JA, Jones KL, Boys CJ, Lim KO, Riley EP, Sowell ER; CIFASD.. Functional connectivity abnormalities and associated cognitive deficits in fetal alcohol Spectrum disorders (FASD). Brain Imaging Behav. 2017 Oct;11(5):1432-1445. doi: 10.1007/s11682-016-9624-4. PubMed PMID: 27734306; PubMed Central PMCID: PMC5389933.
Complete	Suttie M, Wetherill L, Jacobson SW, Jacobson JL, Hoyme HE, Sowell ER, Coles C, Wozniak JR, Riley EP, Jones KL, Foroud T, Hammond P; CIFASD.. Facial Curvature Detects and Explicates Ethnic Differences in Effects of Prenatal Alcohol Exposure. Alcohol Clin Exp Res. 2017 Aug;41(8):1471-1483. doi: 10.1111/acer.13429. Epub 2017 Jul 10. PubMed PMID: 28608920; PubMed Central PMCID: PMC5563255.
Complete	Gross LA, Moore EM, Wozniak JR, Coles CD, Kable JA, Sowell ER, Jones KL, Riley EP, Mattson SN; CIFASD.. Neural correlates of verbal memory in youth with heavy prenatal alcohol exposure. Brain Imaging Behav. 2017 Jun 27. doi: 10.1007/s11682-017-9739-2. [Epub ahead of print] PubMed PMID: 28656347; PubMed Central PMCID: PMC5745322. [Epub ahead of print]

***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.

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 will also re-contact 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 0.25mL aliquot of plasma will be shipped under the appropriate materials transfer agreement to Dr. Weinberg's lab at the University of British Columbia.

If a project you were the PI on was part of Phase III of CIFASD, use this space to update the Advisory Committee on any new progress not previously reported.

The training video created by Dr. Jones during Phase III of CIFASD and titled "Diagnosis of the Fetal Alcohol Syndrome," has been viewed by 92 people on YouTube. Currently, 28 health care providers have completed the survey that follows the video. Analysis of the results from these surveys will occur in the next couple of months.

Principal Investigator: Christina D. Chambers
Institution: University of California, San Diego
CIFASD4 Project Title: Early Predictors of FASD in Ukraine
Grant Number: U01AA014835

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

The current study, CIFASD Phase IV (CIFASD4), 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?

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 neurobehavioral testing at 7-10 years of age for a subset of older children in the cohort. Foreign IRB approval has just been obtained for the final protocol. Training on the school age testing battery took place at both Ukraine sites in Fall of 2017. Testing materials have been purchased and delivered/shipped to the sites.

Immune Function Biomarker

1) Major Activities. Based on the hypothesis that PAE impacts immune function, peripheral immune status was investigated in collaboration with Dr. Weinberg (Aim 1.c.). A comprehensive examination of cytokines, chemokines, angiogenesis, and vascular markers in plasma samples from 18 alcohol-exposed and 13 unexposed children (2 – 3.5 years old) was performed. Cytokine assays were performed using the Meso Scale Discovery (MSD) human biomarker kit, which allows for the measurement of 40 immune-related proteins.

2) Specific Objectives. To determine if inflammatory biomarkers in children are correlated with PAE and/or FASD.

3) Significant Results, Developments or Conclusions. Our preliminary results show differential immune profiles in alcohol-exposed compared to unexposed children. Specifically, alcohol-exposed children showed higher levels of C-reactive protein (CRP), an acute-phase protein and general marker of inflammation. Levels of IL-5 were also elevated in alcohol-exposed children, which has been previously associated with allergic disorders, as well as asthma. Finally, as cytokines do not operate independently, ongoing analyses are utilizing data reduction techniques in order to identify the impact of PAE on the complex and interconnected networks of cytokines. Taken together, changes in the peripheral immune balance during critical developmental periods may underlie some of the long-term effects of PAE on cognitive, physiological, and immune function, as well as the increased risk for mental health disorders later in life.

MicroRNA Biomarker in Mothers

1) Major Activities. In collaboration with Dr. Rajesh Miranda's lab, we previously identified 11 miRNAs in maternal plasma that were significantly elevated and reasonably predictive of FASD. IPA™ pathway overrepresentation analysis indicated the identified miRNAs influence pathways related to fetal and placental growth and maturation, including the epithelial-mesenchymal transition (EMT) pathway. Our prenatal alcohol exposure (PAE) mouse model also showed impairment of the EMT pathway in placenta, with significant elevation in transcript and protein levels of the epithelial E-Cadherin marker.

2) Specific Objectives. To investigate whether the identified miRNAs mediate the effects of ethanol on the placenta, we assessed their effects on BeWO and HTR8 human trophoblast cell lines.

3) Significant Results, Developments or Conclusions. Overexpression and knockdown of the selected microRNAs significantly reduced growth, retarded cell cycle progression, and reduced the migratory/invasive capacity of both cell lines, pointing to their role in modulating the placental growth and invasion deficits seen in PAE. Subsequent analysis of EMT pathway members, following selected miRNA overexpression, showed impairment of EMT pathway in both HTR8 and BeWO cells with increased expression of E-Cadherin in BeWO cells and reduced Vimentin expression in HTR8 cells. Overexpression of the selected miRNAs prevented the reduction in E-Cadherin expression following forskolin-induced BeWO syncytialization (maturation) without interfering with syncytialization-dependent increases in placental hormone expression. Taken together, these data suggest that maternal circulating miRNAs may affect placental growth and invasion, and, following PAE, selectively interfere with the EMT pathway, thereby contributing to the pathology of FASD.

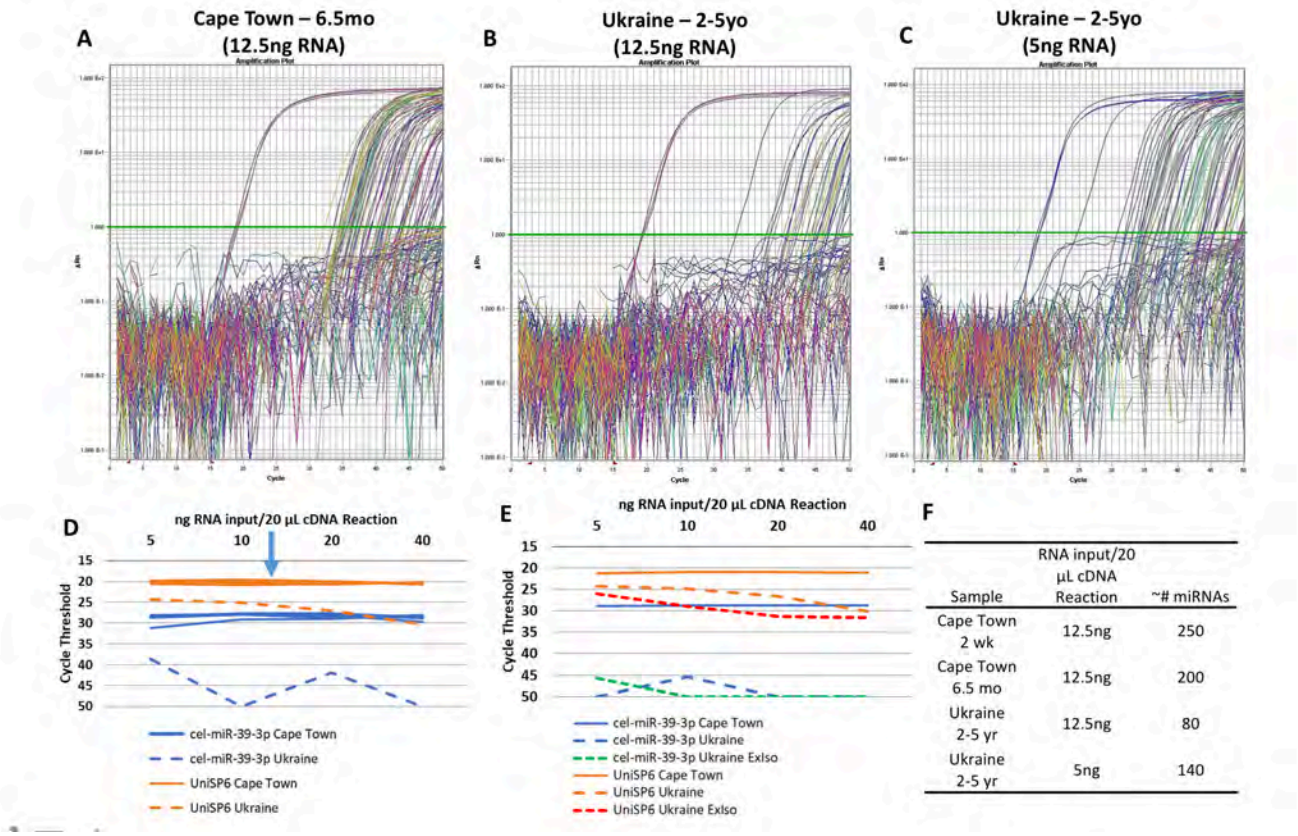
MicroRNA Biomarker in Children

1) Major Activities. miRNA expression in plasma samples from 45 children (22 alcohol exposed affected and 23 unaffected or unexposed) who were 2-5 years of age have been analyzed (Aim 1.a.).

2) Specific Objectives. To determine if the miRNA expression patterns and pathways in children are predictive of PAE and/or FASD.

3) Significant Results, Developments or Conclusions. Examination of the raw amplification data revealed that the number of miRNAs amplified in the Exiqon miRCURY LNA™ Universal RT microRNA PCR panels was decreased compared to infant samples from 2 weeks to 6.5 months of age that Dr. Miranda's lab has assessed from the Cape Town region in South Africa (See panel A, B below). Analysis of the data from Cape Town suggested that miRNA quantity may decline over development,

as 2 week samples had ~250 circulating miRNAs identified while 6.5 month samples had ~200, which was higher that the ~80 seen with the Ukraine child sample (see panel F below). To further assess if the decrease in circulating miRNA number in the Ukraine child samples was age-related or due to experimental conditions, we investigated the possible presence of PCR inhibitors in the child samples. Two RNA spike-ins were included during cDNA synthesis, cel-miR-39-3p and UniSP6 (100-fold higher concentration than cel-miR-39-3p), to assess the possible inhibition of the reverse transcription of both highly expressed (UniSP6) and lowly expressed (cel-miR-39-3p) miRNAs. We found decreased expression of these spike-ins with increasing levels of RNA input, particularly of the lower expressed cel-miR-39-3p, indicating that cDNA synthesis was being inhibited (see panel D below) (RNA input of 12.5 ng/20 µL reaction shown by blue arrow). We further examined if the Exiqon miRCURY Biofluids miRNA isolation kit would reduce the presence of inhibitors to a greater degree than the Qiagen miRNeasy isolation kit which we had previously used successfully with both Cape Town infant samples and Ukraine maternal samples. We found slight improvement in the expression of low level miRNAs (as seen by the greater expression of low level cel-miR-39-3p spike-in) when using the Exiqon isolation kit (see panel E below). Additionally, reduction of the total level of RNA input (to 5ng/20uL cDNA reaction) resulted in increased number of miRNAs detected (from 83 to 141, 70% more) (see panels C, F below). While the identity of the inhibitor in this child samples is currently unknown, we are confident that decreased RNA input and isolation with the Exiqon kit will provide a robust sample of circulating miRNAs in these children to analyze for indicators of PAE.



Sample	RNA input/20 µL cDNA Reaction	~# miRNAs
Cape Town 2 wk	12.5ng	250
Cape Town 6.5 mo	12.5ng	200
Ukraine 2-5 yr	12.5ng	80
Ukraine 2-5 yr	5ng	140

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 completed the first analysis of trajectories for prediction of neurodevelopmental performance incorporating risk and resilience factors into individual prediction models.

2) Specific Objectives. The objective of this analysis was to develop a flexible and parsimonious model that can assign risk scores to individual children at a given age in development based on readily

available data in a routine clinical setting. Growth was the primary predictor. Data used in this analysis consisted of 742 live born singleton infants for whom at least 1 pre- or postnatal growth measure was available. This included 573 children with 874 ultrasound weight measures, 618 children with 1,159 ultrasound head circumference measures, 741 children with 2,189 postnatal weight measures, 742 children with 1,938 postnatal head circumference measures, and 742 children with 2,202 postnatal height measures. These data were used to develop individual growth curves for each child using a novel fast covariance estimation (FACE) method. Individual growth curve data was used in combination with selected clinical variables to predict neurobehavioral performance on the Bayley Scales of Infant Development at 12 months of age.

3) Significant Results, Developments or Conclusions. The individual growth curves for the subset of 441 singleton infants who had completed the Bayley Scales of Infant Development testing at 12 months of age (192 born to mothers in the alcohol-exposed group and 249 born to mothers in the low-unexposed group) were combined with clinical variables that were screened for their contribution to improvement in the prediction models. Among the 192 alcohol exposed infants, the area under the curve (AUC) for the prediction models of the dichotomized (<85 or not) Bayley Mental Development Index (MDI) and the Psychomotor Development Index (PDI) were 0.81 and 0.85, with out-of-sample AUC of 0.76 and 0.81, respectively. The explained variation for the continuous MDI and PDI were 37% and 33%, respectively. The prediction was less satisfactory among the alcohol unexposed infants. These findings suggest that individual prediction models can be used in clinical practice to assign risk scores those children prenatally exposed to alcohol who are at high risk of delay as well as children less likely to be impaired as they develop. Next steps are to extend the prediction models to preschool and school age neurodevelopmental endpoints, and to incorporate biomarkers from Aim 1 to evaluate the marginal benefit of these more costly measures.

Aim 3. Collaborate with others in the CIFASD consortium

1) Major Activities. During this reporting period, we provided 31 biological samples from children in 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.

During this reporting period, we provided data from the Ukraine cohort to U01 PI Mattson to test/adapt the FASD Decision Tree.

During this reporting period, we have worked with UH2 PI Torii to further modify the blood sample collection procedure to meet their requirements. Previously banked samples are not amenable to the analysis they propose. Although new infant samples are expected to be available by 2019, by agreement with Dr.Torii, samples using the new procedure are being collected from 7-10 year old children in the cohort. We expect to receive a shipment of these samples in 2018.

During this reporting period, we have discussed with Dr. Hammond the procedure for ultrasound data. At this point, it appears that no specific data points or views are identified, and Dr. Hammond would prefer a full recording of all ultrasound scans for exploratory analysis. The feasibility of doing this is being discussed with the collaborators in Ukraine.

The 3D camera remains at one site in Ukraine and will be used as children are seen for other study visits.

2) Specific Objectives. To provide samples and data to other investigators.

3) Significant Results, Developments or Conclusions. See Aim 1.c. results above.

Table of enrollment and samples for CIFASD4

Category	N
New Data (Aim 1)	
Mothers	
Enrolled	Moderately/heavily exposed= 0 (goal 120 Yrs. 1-3) Low/unexposed= 0 (goal 80 Yrs. 1-3)
2D ultrasound on new mothers	Moderately/heavily exposed =0 Low/unexposed= 0

Category	N
5 mL blood samples collected	Moderately/heavily exposed at enrollment= 0 Low/unexposed at enrollment= 0 Moderately/heavily exposed at 3rd trimester= 0 Low/unexposed at 3rd trimester= 0
Infants	
Enrolled	Female = 0 Male = 0 Total = 0 (goal 180)
Dysmorphology exams	Completed exams = 0 (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 = 0 School age 7-10 year olds low/unexposed = 0
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 = 0 School age 7-10 year olds low/unexposed = 0
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 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

Category	N
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 Hammond	2D ultrasound = 0
UH2 PI Torii	Infant blood samples Moderately/heavily exposed new infants= 0 Low/unexposed exposed new infants = 0 Blood samples Moderately/heavily exposed children 7-10 years of age = 0 Low/unexposed exposed children 7-10 years of age = 0

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
Since June 1, 2017, one 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 was successful in being awarded an F31 using this project as the basis.

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. Data from this study have been presented at RSA.

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: initiate recruitment of new mothers; capture bio samples; test 7-10 year olds
Aim 2: expand prediction models to preschool testing outcomes; incorporate COR, and existing maternal biomarkers data on subset
Aim 3: continue to work with other investigators in the Consortium

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

Sowell KD, Uriu-Adams JY, Van de Water J, Chambers CD, Coles CD, Kable JA, Yevtushok L, Zymak-Zakutnya N, Wertelecki W, Keen CL and the CIFASD. Implications of altered maternal cytokine concentrations on infant outcomes in children with prenatal alcohol exposure. *Alcoho*l, In Press.

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

Bodnar TS et al. Altered maternal immune networks are associated with adverse child neurodevelopment: Impact of alcohol consumption during pregnancy. Submitted to *Biological Psychiatry*. January 2018.

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

Coles, CD, Kable, JA, Yevtushok, L, Zyma-Zakutnya, N, Wertelecki, W., Chambers, CD, and the CIFASD. (in preparation) Risk factors for preterm birth in the Ukrainian alcohol and nutrition cohort. In preparation.

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. In preparation.

***Poster Abstracts and Presentations**

Abstracts

Coles, CD, Kable, JA, Granovska, IV, Pashtepa, AO, Wertelecki, W, Chambers, CD, & the CIFASD (2017). Using a nonverbal assessment battery to identify cognitive effects of alcohol and modifying factors cross culturally in Ukrainian preschool children. Special Issue: Abstracts from the 40th Annual Scientific Meeting of the Research Society on Alcoholism, Denver, CO, 41(S1): 60A

Mesa, DA, Kable, JA, Yevtushok, L, Kulikovsky, Y, Wertelecki, W, Coleman, TP, & Chambers, CD (2017). The use of cardiac orienting responses as an early and scalable biomarker of alcohol-related neurodevelopmental impairment in later age children. Special Issue: Abstracts from the 40th Annual Scientific Meeting of the Research Society on Alcoholism, Denver, CO, 41(S1): 57A.

Bodnar TS, Raineki C, Wertelecki W, Yevtushok L, Plotka L, Zymak-Zakutnya N, Wells A, Honerkamp-Smith G, Coles CD, Kable J, Chambers CD, Weinberg J, and the CIFASD. Cytokine disturbances associated with prenatal alcohol exposure in children: implications for health and development. Submitted abstract to 41st Annual RSA Scientific Meeting, San Diego, CA, June 2018. Submitted

Tseng AM, Balaraman S, Allan AM, Chambers C, Miranda RC. Endocrine miRNAs in pregnant women, predictive of FASD infant outcomes, control placental trophoblast growth, survival and maturation. Submitted to RSA 2018.

Presentations

Coles, CD, Kable, JA, Granovska, IV, Pashtepa, AO, Wertelecki, W, Chambers, CD, & the CIFASD. Using a nonverbal assessment battery to identify cognitive effects of alcohol and modifying factors cross culturally in Ukrainian preschool children. (Poster) 40th Annual Scientific Meeting of the Research Society on Alcoholism, Denver, CO, June 24-28, 2017.

Mesa, DA, Kable, JA, Yevtushok, L, Kulikovsky, Y, Wertelecki, W, Coleman, TP, & Chambers, CD. The use of cardiac orienting responses as an early and scalable biomarker of alcohol-related neurodevelopmental impairment in later age children. (Poster) 40th Annual Scientific Meeting of the Research Society on Alcoholism, Denver, CO, June 24-28, 2017.

Symposia

Dunty W and Chambers CD Co-Chairs: Identifying prenatal alcohol-affected individuals early in life: the use of novel screening tools and methodologies in human populations. Submitted for RSA 2018.

Charness M and Riley E Co-Chairs: Predicting outcomes of fetal alcohol exposure in the CIFASD cohort. Submitted for RSA 2018.

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

This project is closely tied to the Weinberg U01, the Torii UH2, and will be providing 2D ultrasound for pregnant women to the Hammond U01. We have worked directly with Mattson U01 to provide test data sets from the existing Ukraine cohort archive and from our NIAAA-funded CoFASP prevalence study to validate/modify the Decision Tree. We will also work with Dr. Mattson in our currently proposed Aim 2.a. to validate the risk/resilience profiles developed in the Ukraine cohort in the retrospective dataset of U.S. children from CIFASD II and III. Additional collaborations may include Foroud U01-genetics, Blanchard UH2-microbiome, and mobile technology tools for capturing data as these are developed.

If a project you were the PI on was part of Phase III of CIFASD, use this space to update the Advisory Committee on any new progress not previously reported.

Progress using CIFASD II and III data reported above.

Principal Investigators: Claire D. Coles; Therese Grant (Subcontract)
Institutions: Emory University School of Medicine; University of Washington (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. Develop methods for studies; Establish collaboration between sites and with Dr. Weinberg's lab. Develop data collection and storage protocols and methods.

2) Specific Objectives.

- Hired and trained staff for recruitment.
- Secured all the Human Subjects approvals.
- Arranged the material transfer agreements.
- Developed recruitment materials and printed them.
- Obtained programs for subject identification and tracking and trained staff on these; Began process of finding accurate locations information for subjects.
- Reviewed all the measures, discussed them among the 3 sites that will be using them and ordered all the materials needed for the length of the project.

- Developed a health survey that can be completed in multiple medias (e.g., on-line; via phone, telephone, in-person). Piloted survey in Atlanta.
- Written recruitment and data collection protocols.
- 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.

3) Significant Results. None as yet.

4) Key Outcomes and Other Achievements. Developed Adult Health Survey and prepared draft of administration manual (H3). No other outcomes as yet.

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 at this time.

B.5 How have the results been disseminated to communities of interest? No results to disseminate at this time.

B.6 What do you plan to do during the next reporting period to accomplish the goals? During the next six months, we will initiate recruitment and identify any problems in the protocols and methods. We hope to have recruited 30 individuals at each site by July 2018.

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.

We are also considering how to most effectively describe the participants' physical status, including dysmorphology. We are talking with Dr. Hammond regarding the analysis of 2-D photographs. We anticipate that we will develop a study design in the next 6 months that can be implemented in the Tier 2 sample. This will require the development and implementation of a standard method for obtaining the 2-D photographs that is agreed on across sites.

***Publications [Accepted & In Press]** Not as yet.

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

***Poster Abstracts and Presentations**

Coles, CD, Grant, T, Weinberg, J. Mapping the Undiscovered Country: Physical and Mental Health in Adults with FASD. Abstract accepted for 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.

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 reach into middle adulthood. This will be the first study to describe the impact of prenatal alcohol exposure on adult functioning in a longitudinal sample of adults whose prenatal exposure can be documented or who were diagnosed as children. In order to carry out this project, we will be collaborating 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 will be working closely with Dr. Weinberg in Vancouver who is planning not only to analyze samples that we will provide to her but also to institute a parallel study of adults in Canada using the same protocol and measures. This collaboration will increase our sample size and generalizability substantially. Working with Peter Hammond, we are also planning to develop methods for analysis of 2-D photographs of adults taken in all 3 sites. Finally, we will work with the Informatics Core to record and archive these data.

Principal Investigator: Tatiana Foroud
Institution: Indiana University
CIFASD4 Project Title: Dissecting the Genetic Contributions to Fetal Alcohol Spectrum Disorders
Grant Number: U01AA026103

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

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	50%
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	0%
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	0%
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	0%
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	0%
b. Support the review of requests for CIFASD data from external researchers and provide de-identified data to approved researchers.	03/31/2022	10%
c. Maintain an online CIFASD volunteer registry to consent subjects interested in participating in future research studies.	03/31/2022	50%

Cohesiveness within CIFASD: This cooperative agreement will have close collaborations with other projects in the proposed 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/Torii). 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.

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

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.2 What was accomplished under these goals?

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

Aim 1: The emphasis has been on developing the protocol for the new online recruitment study, including all consents/assents, case report forms, and data collection protocols. The IRB protocol will be submitted the second week of January and we expect to have the website fully functional and able to consent participants by May 2018.

Aim 2: The emphasis has been 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: The emphasis has been on creating an additional data collection tool to accommodate school-age Ukrainian cohort and facilitating analysis of the maternal alcohol use via visualizations of its trajectories. In addition, we streamlined the process of the review of requests for CIFASD data from external researchers, and finalized the application form for an online CIFASD registry to assist the internal CIFASD researchers in contacting subjects interested in participating in future CIFASD research studies. We continue to support collection and tracking of research data into the central repository, with the goal of making the data available to CIFASD and external researchers.

3) Significant Results.

Aim 1: For this new grant, a new Indiana University IRB protocol was developed. This has included separate consent and assent documents. These will be completed online through the web portal. To help families participate in this study online, a video assent will be prepared once the language of the assent has been approved by the Indiana University IRB. A website will be finalized once the Indiana University IRB approves the draft content. A series of case report forms will be finalized in RedCap and will collect information on: participant demographics, previous medical evaluations for FASD, prenatal alcohol and other drug exposure, as well as other relevant information. A protocol was developed by Peter Hammond (U01AA014809: Image Analysis of Neurofacial Effects of Prenatal Alcohol Exposure) for the collection of 2D facial images and the directions are being prepared into a video that participants can view prior to taking the pictures with their mobile device. Directions are already prepared that are included with the saliva kit that is sent to the home of the participant. Everything is on track for the webportal to be live in May 2018 and the first participants consented and enrolled.

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. We have generated WES in two rounds. Due to the declining costs, we have obtained WES from two different laboratories. To ensure that we can combine results across the two laboratories, we included 10 subjects in both rounds of WES. In total we have obtained CIFASD WES from 285 samples.

Data from the two labs were called jointly using GATK best practice to minimize possible lab effects, and then extensive quality control was performed. The concordance rate of the 10 samples typed in both labs was 93.6%. Data for the 153 individuals who were also in the genome-wide association analysis were compared, with a concordance rate of 99.0%. Gender and cryptic relationship were also examined. The final confirmed WES data set consists of 260 samples with prenatal alcohol exposure: 60 with FAS (23.1%), 108 with a deferred status (41.5%), and 92 a diagnosis of no FAS (35.4%).

Our analyses are designed to compare two groups of subjects with prenatal alcohol exposure, one with FAS and the other with no evidence of FAS who have been classified as ‘no FAS’. Whole exome sequencing is designed primarily to test the effect of rare variants in the coding region of genes. We are focusing our initial analyses on 60 FAS individuals and 92 individuals designated as “no FAS”. We are using the statistical analysis program, RVTEST, to perform a gene based test of the effect of rare variants. We are not limiting our analyses to a single racial/ethnic group, so we are including 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 are first evaluating 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*.

Aim 3: In collaboration with Drs. Coles and Chambers, we developed a new collection tool to accommodate school-age Ukrainian cohort which is aimed at collecting Neurobehavior data. In support of Dr. Chambers' analysis of the Ukrainian cohort, we created an extensive visualization of the maternal alcohol use trajectories using Tableau platform. Nine dashboards depict data from various data sources including Dysmorphology, Infant and Preschool Neurobehavior, Alcohol and Control and Follow-up/Outcome subtests consisting of ~200 dimensions and measures. Dr. Chambers team has access to these dashboards via the Tableau server at Indiana University. We are working on creating Data Cookbook metadata documentation to ensure transparency of dashboard calculations and filters.

The Data Access Committee streamlined the process of the review of requests for CIFASD data from external researchers by implementing a data request form. We are working with Dysmorphology, Genetics, Brain Imaging, and Neurobehavior Cores PIs to improve the description and cleanliness of the Phase 2 data available for the external researchers.

We continue to maintain the online CIFASD volunteer registry and added a request for access form to assist the internal CIFASD researchers in contacting subjects interested in participating in future CIFASD research studies. By January 2018, the registry collected 264 records with 176 potential volunteers from US and Canada including which includes self-volunteers (56) and volunteering their children (120).

The online CIFASD volunteer registry is approximately equal proportion of females and males (Female: 83, 49.4%, Male: 84, 50.0%), Prefer not to Answer (1, 0.6%). The volunteer registry is also diverse: American Indian or Alaska Native (16, 9.1%), Asian (8, 4.5%), Native Hawaiian or Other Pacific Islander (0, 0.0%), Black or African American (18, 10.2%), White (133, 75.6%), Other (19, 10.8%), Prefer not to Answer (1, 0.6%)

Table 1: Age of the online CIFASD volunteer registry

				Percentile						
Min	Max	Mean	StDev	**0.05**	**0.10**	**0.25**	Median	**0.75**	**0.90**	**0.95**
1.00	83.00	22.23	18.37	5.00	7.00	10.00	14.00	25.00	52.70	62.35

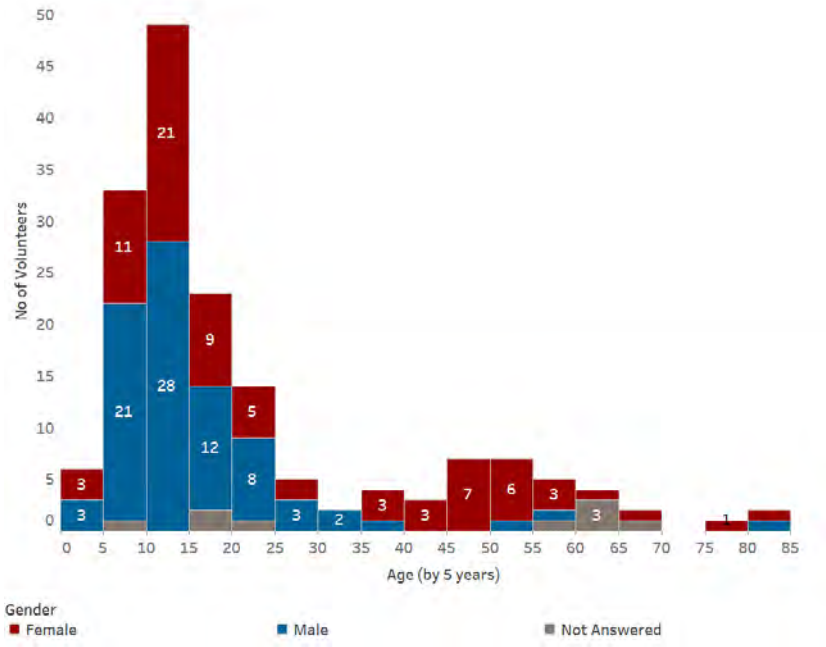


Figure 1: Age and Gender Distribution of online CIFASD volunteer registry

4) Key Outcomes and Other Achievements.

Multiple projects could interact and feed participants to each other. One approach that has been discussed is the use of a global unique identifier (GUID), which would assign the same GUID to a participant participating in multiple projects.

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?

Professional Development: Leah Wetherill and Elizabeth Rowe will both attend the CIFASD meeting in February as well as the Research Society on Alcoholism (RSA) conference in June (including the FASD Satellite meeting). This provides both of them with increased knowledge in the field of FASD in general, and more specifically with new research developments. Elizabeth Rowe has submitted an abstract for a poster presentation at RSA which will allow her to present the web portal of this new project. Tatiana Foroud is part of a submitted symposium at ISBRA which will highlight results from the genetic analysis.

Helen Yezerets will attend the annual CIFASD meeting. Ms Yezerets successfully completed a course of Statistical Inference and its Application with R through the Master in Data Science program at IU. She also participated in R/Shiny training for researchers. She continued improving her understanding of the Tableau platform by virtual attendance on the Tableau 2017 conference. Finally, Helen is taking a Machine Learning class through Coursera platform to solidify her knowledge in this area.

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. We have chosen to delay specific outreach until the web portal is fully deployed and we can direct communities of interest to the site to learn more about the study and also consent and participate.

With Specific Aim 3, we are working on improving the dataset description on http://cifasd.org/data-sharing/ page to facilitate the process of requesting and accessing the CIFASD data from phase 1 and 2 for the external researchers.

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

Aim 1: The focus in the next year will be deployment of the website, outreach to communities of interest and FASD support groups in collaboration with the Outreach/Dissemination Core, enrollment of new CIFASD participants, and coordination with other CIFASD projects to co-enroll participants. We will carefully review the accrued data to identify any changes needed in the website, consenting or case report forms. We will also review collection protocols (2D facial image and saliva) to determine if any changes are needed in these protocols. During the coming year, we will be working with the Mattson project (U01AA014834: A Multisite Neurobehavioral Assessment of Fetal Alcohol Spectrum Disorders) in the implementation of the FASD Online Neurobehavioral Screen (FONS) on this project's web portal.

Aim 2: As we generate results from our candidate gene based analyses, we will share them 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. We will be starting next genomewide gene-based analyses. Power for this broader analysis is more modest, so we will consider these results preliminary as we continue to increase the sample size for these analyses.

Aim 3: In the upcoming year we will implement the upload/query and download functionality in the central repository for the school-age Ukrainian cohort. We plan to work with Drs. Coles, Chambers and Weinberg on implementation a central repository storage for the maternal and infant miRNAs, cytokine profiles and epigenetic factors when those data become available. We will continue providing visualizations of the data from multiple data sources. We will finalize the improvements for phase 1 and 2 data and the respective metadata and continue to review requests for CIFASD data from external researchers. We will reach out to the internal CIFASD project PIs to facilitate contacting volunteers interested in participating in future CIFASD research studies through the CIFASD registry.

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

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

***Poster Abstracts and Presentations**

Rowe, E., Wetherill, L., Foroud, T., CIFASD. Dissecting the Genetic Contributions to Fetal Alcohol Spectrum Disorders (DIG FASD): A CIFASD Consortium Study. Poster abstract submitted to the Research Society on Alcoholism 41st Annual Scientific Meeting (San Diego, CA; June, 2018)

Wetherill, L, Mattson SN, Foroud T, Goodlett C, CIFASD, COGA. Effect of prenatal alcohol exposure and parental alcohol dependence on risk of externalizing disorders in COGA and CIFASD samples. Poster abstract submitted to the Research Society on Alcoholism 41st Annual Scientific Meeting (San Diego CA; June, 2018).

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

Table 1: Summary of CIFASD Interactions with the Genetics project

	PI	CIFASD Resource/Project	Interaction
U24	Riley	Administrative	The Genetics Project will report progress to the Administrative Resource and interact with the Administrative Resource leader to facilitate collaborative interactions and guidance on research questions and priorities. The Informatics Group in the Genetics Project creates monthly reports of the central repository utilization and statistics as well as CIFASD registry statistics. In addition, it provides assistance to potential users of the CIFASD registry, and fields data requests from external researchers.
U24	Jones	Dysmorphology	The Genetics Project will work with the Dysmorphology Resource to confirm documentation provided by individuals through the web portal regarding past assessments for FASD. The Dysmorphology Resource will inform parents of children with prenatal alcohol exposure from the Research Registry at Rady Children's Hospital of the Genetics Project web portal.
U24	Donaldson	Outreach/Dissemination	Research findings will be shared by the Genetics Project with the Outreach/Dissemination Resource to raise public awareness of FASD. The Outreach/Dissemination Resource will direct individuals to the Genetics Project web portal for recruitment.
U01	Hammond	Image Analysis of Neurofacial Effects of Prenatal Alcohol Exposure (Imaging Analysis Project)	The Genetics Project will work with the Imaging Analysis Project to develop a video describing the protocol for taking a frontal and profile 2D facial images. The Genetics Project will share 2D facial images directly with this project. The Informatics Group in the Genetics Project will work with the Imaging Analysis Project to finalize the phase 1 and 2 measurements and metadata and provide respective descriptions on the http://cifasd.org/data-sharing/ page.
U01	Mattson	A Multisite Neurobehavioral Assessment of Fetal Alcohol Spectrum Disorders (Neurobehavioral Project)	The Genetics Project will work with the Neurobehavioral Project to identify neurobehavioral assessments that can be administered online for individuals of a wide age range. De-identified data from these assessments will be provided to the Informatics Resource where other projects can download these data. In addition, the Informatics Group in the Genetics Project will work with the Neurobehavioral Project to finalize the phase 1 and 2 measurements and metadata and provide respective descriptions on the http://cifasd.org/data-sharing/ page.

U01	Petrenko/ Tapparello	Development and Evaluation of an Evidence-Based Mobile Health Caregiver Intervention for FASD (Intervention Project)	The Genetics Project will identify eligible individuals recruited through the web portal who will be invited to participate in the Intervention Project. The Genetics Project will work with the Intervention Project to develop a video consenting process for subject enrollment. The Informatics Group in the Genetics Project will reach out to the Intervention Project to provide access to the eligible volunteers in the CIFASD research studies.
U01	Eberhart/ Parnell	Characterizing the Genetics of FASD in Complementary Mouse and Fish Models (Animal Genetics Project)	The Animal Genetics Project and Genetics projects will share with each other candidate genes identified in their studies. The groups will seek to replicate the findings of the other using complementary models.
U01	Weinberg	Immune Dysregulation in FASD: Programming of Health and Neurobehavioral Outcomes	The Genetics Project will share genes identified in the immune pathway to the Immune Project to explore if these genes moderate the immune response to alcohol-induced dysregulation. The Health and Neurobehavior Outcomes Resource will direct individuals to the Genetics Project web portal for recruitment. The Informatics Group in the Genetics Project will develop a central repository for the biomarker of inflammation and cytokine profiles provided by the Health and Neurobehavior Outcomes Resource
U01	Hashimoto/ Torii	Biomarker for Intellectual Disability in Children Prenatally Exposed to Alcohol	Genes identified in the Epigenetic Biomarker Project will be tested in the Genetics Project.
U01	Coles	Fetal Alcohol Spectrum Disorders in Adults: Health and Neurobehavior	The Genetics Project will identify eligible individuals recruited through the web portal who will be invited to participate in the Health and Neurobehavior Project. The Health and Neurobehavior Outcomes Resource will direct individuals to the Genetics Project web portal for recruitment. The Informatics Group in the Genetics Project will create a central repository for the school-age Neurobehavior components and direct potential volunteers to the Genetics Project web portal for recruitment.
U01	Wozniak	Multi-modal Connectivity Methods for the Validation of Fetal Alcohol Spectrum Disorder Diagnostic Criteria	The Genetics Project will identify eligible individuals recruited through the web portal who will be invited to participate in the Multi-modal Connectivity Methods Project. The Multi-modal Connectivity Methods Resource will direct individuals to the Genetics Project web portal for recruitment.
U01	Chambers	Early Predictors of FASD in Ukraine	The Early Predictors of FASD in Ukraine Resource will identify individuals who are English-speaking and direct them to the Genetics Project web portal for recruitment. The Informatics Group in the Genetics Project will work with the Early Predictors Project to create a miRNA central repository and provide updates to the visualizations of the maternal alcohol use trajectories.

If a project you were the PI on was part of Phase III of CIFASD, use this space to update the Advisory Committee on any new progress not previously reported. Tatiana Foroud was a multiple PI with Peter Hammond on a previous CIFASD collaborative grant (U01AA014809: 3D Facial Imaging in FASD). The analysis of the genetic data generated as part of that project are now included in the goals of this new project. Analysis of the facial imaging data continues as part of Peter Hammond's project (U01AA014809: Image Analysis of Neurofacial Effects of Prenatal Alcohol Exposure). In Phase 3, Barnett as PI of the Informatics Core (U24AA014818) led the development of the CIFASD Data Access Committee, which now has established policies and procedures to provide CIFASD data to external investigators, which continues into this phase. The Informatics Core also piloted a volunteer registry that continues into Phase 4. The previous Informatics Core is now part of the Genetics Project.

Principal Investigator: Peter Hammond
Institution: University of Oxford
Title: Image Analysis of Neurofacial Effects of Prenatal Alcohol Exposure
Grant Number: U01AA014809

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

Aim 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.

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

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

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?

Aim 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, 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:

- CIFASD online recruitment
  - lay access to web portal
  - 2D image
  - no immediate user feedback
  - later offline analysis by researcher
- FASD clinic
  - smartphone
  - 3D image
  - semi-automated analysis on laptop
  - immediate diagnostic feedback to clinician.

3D FACIAL ANALYSIS

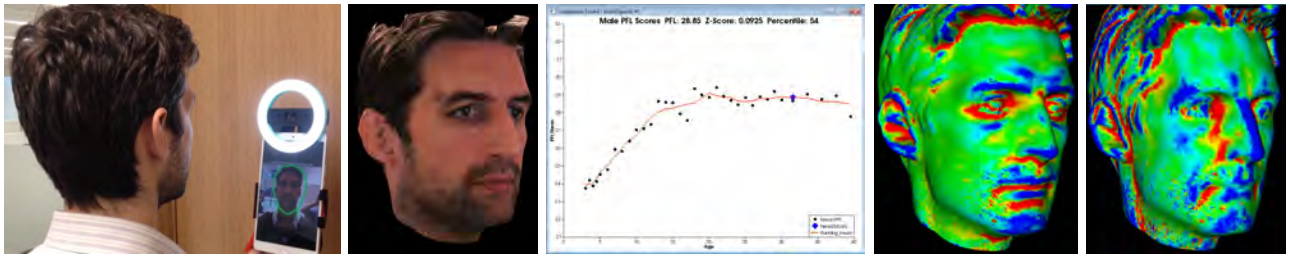
Semi-automated face screening in the UK FASD clinic of Dr Raja Mukherjee

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. Currently, Dr Mukherjee uses the Astley-Clarren software from Washington University for a 10-15 minute assessment of the face in the first 2 hour session on the first of two days of assessment. With local funding, Dr Mukherjee has purchased the Canfield H1 hand-held 3D camera (used now by several CIFASD collaborators) and has collected 30 or so 3D facial images of children and adults. Mike Suttie has demonstrated our face screening software at the clinic with Dr Mukherjee in order to get feedback on both user interface and the level of analysis required. Some functionality of the face screening tool is illustrated below in the description of its use with the Bellus smartphone/tablet camera. A comparison of the face analyses produced semi-automatically by the University of Washington system and our face screening tool for the 30 or so cases recruited is imminent. It is hoped that some conclusions will be available for the CIFASD face-to-face meeting of February 2018.

Automated 3D landmarking using Bellus smartphone/tablet based 3D camera

The Bellus 3D camera (obtained with administrative core funding for testing in Oxford) uses a small attachment to a smartphone or tablet to capture 3D facial photographs (Fig. 1a) – specifically for 3D

"selfies". The associated commercial software supports automated annotation of the image with 3D anatomical landmarks (Fig. 1b). The developer version that CIFASD has bought gives direct access to the landmarks, although the particular landmarks used are built-in and currently beyond user selection. The landmarks and the 3D facial image can then be exported to produce and/or test face shape models that have been the basis of previous 3D facial analysis in CIFASD2 and CIFASD3. In contrast, although the Canfield H1 hand-held camera also provides automated landmarking its initiation is embedded in software designed for virtual surgery and the landmarks are not so readily available.



a b c d e

Fig 1: a) Bellus camera; b) automatically landmarked 3D image; c) PFL normalized against relevant controls; d) automated production of curl heat map; e) automated production of groove heat map.

The Bellus camera can be configured to save the acquired images and landmarks to a server. We have arranged for our face screening software to access the landmarked image so that without user intervention it can:

- compare palpebral fissure length graphically to norms obtained from public sources (Fig. 1c);
- display heat maps of surface curvature of the face to visualize philtrum smoothness and to some extent malar flattening (Figs. 1d & 1e).

The manual addition of two more landmarks (lower ear attachment points) enables the image to be compared automatically with pre-existing shape models of the full face and facial regions and to be classified on the control-FAS spectrum (Fig. 2).

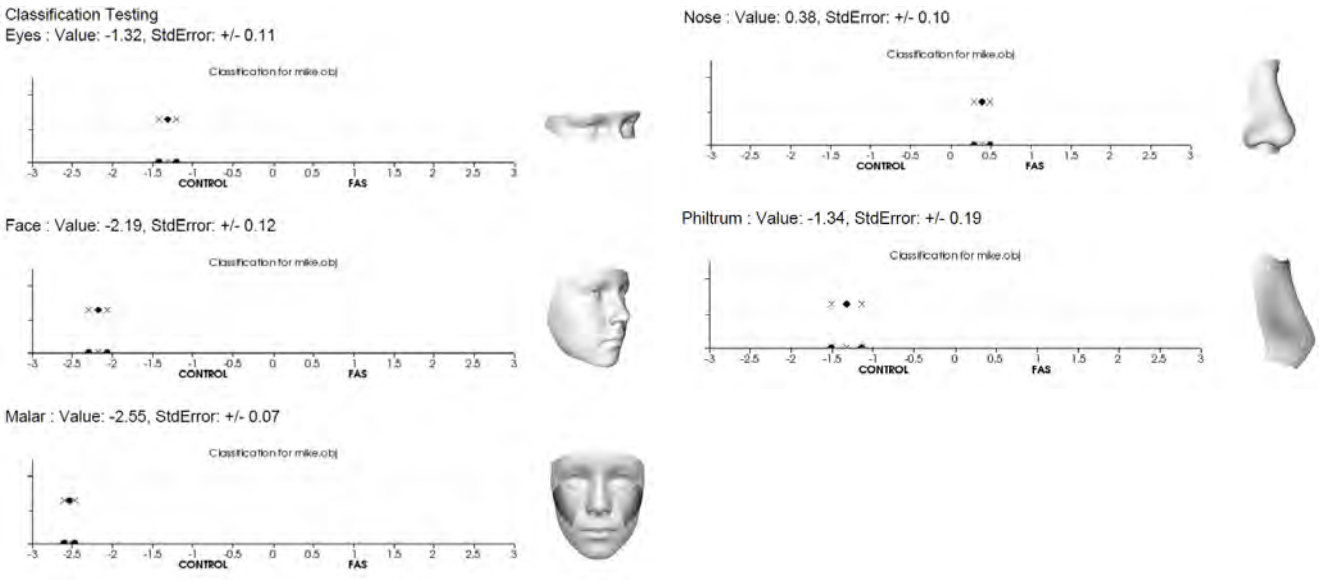


Fig 2: Automated classification of face and face regions on a control-FAS spectrum

2D FACIAL ANALYSIS

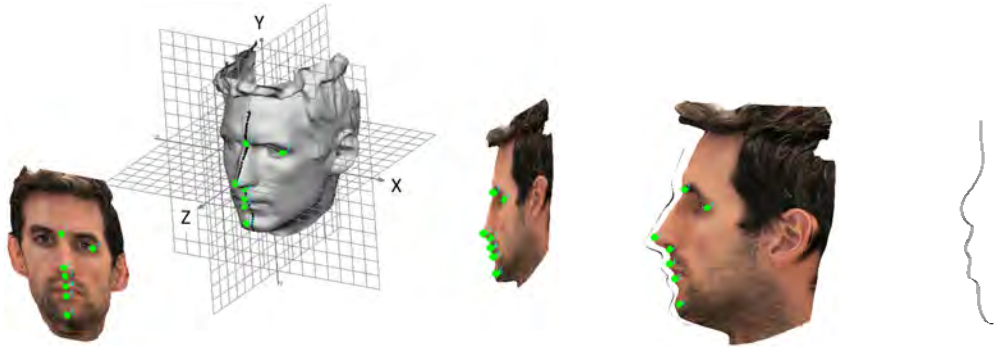
In CIFASD4, 2D facial photographs will be collected through the web-based recruitment portal being produced at Indiana University. Other CIFASD4 collaborators will also be collecting 2D images using conventional cameras. We have worked closely with CIFASD colleagues to produce a protocol for 2D facial photography capturing three views of the face: full portrait; lateral profile; and, three-quarter profile (see APPENDIX). A pair of small adhesive labels will be located on the forehead and cheek to enable subsequent estimates of scale and camera position.

2D images captured in previous iterations of the CIFASD consortia will be useful in terms of testing and developing 2D facial analysis techniques. Obtaining ethics permission is currently delaying their transfer to Oxford for analysis. Oxford colleague Chris Nellaker, a specialist in 2D facial analysis, will become more involved towards the end of year 1 of CIFASD4 (and thereafter) in terms of applying his specific 2D analysis techniques.

New 2D photographs can be projected into a 3D model constructed from existing 3D photographs. Until CIFASD 2D photographs are available, we have used CIFASD 3D images along with an artificial camera model to derive pseudo 2D profile and portrait facial images. From the 2D profiles, we extracted 2D curves so that they can be used within 3D face shape models already developed in CIFASD3 (Fig. 3b). As an interim measure, these images were also manually annotated with a small number of landmarks (see Figs. 3a & 3b).

2D profiles as a pseudo 3D profile model to build a shape model for control-FAS classification

The extracted 2D profile curves can be converted to ribbon-like 3D surfaces (Fig. 3c) 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%. This is impressively close to the clinical concordance achieved previously using 3D image analysis of the surface of the face and selected regions.



a b c

Fig. 3 a) Projection of 2D images into 3D model; b) pseudo profile photograph and extracted profile curve; c) thin ribbon profile used in 3D control-FAS classification

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

Face-brain analysis started in CIFASD3 has continued and a journal manuscript has been submitted. The previous studies involving corpus callosum (CC) have been extended to include the caudate nucleus (CN) and in collaboration with CIFASD behavioural science partners, in particular Dr. Jeff Wozniak, new face-brain correlations have been identified in terms of asymmetry and specific cognitive measures. In particular, we established that

- "facial growth" for FAS, HE and controls is replicated for CN but not CC;
- a subset of HE was identified with FAS-like CN dysmorphism and an average face with FAS-like facial dysmorphism;
- midline facial regions discriminate better when combined with midsagittal profile of CC;
- face with CN identified an HE subset with FAS-like face with brain dysmorphism;

- most facial regions combined with left CN discriminate better than same region with right CN, and with whole CN – suggesting asymmetry of effect;
- for CN: net directional asymmetry in mean control is not seen in mean FAS and is diminished in mean HE; and
- for CN: asymmetry index (Euclidean distance between PCA vector representations of original and mirrored surfaces) correlates with a variety of cognitive measures in exposed individuals (FAS+HE) but not in controls (see Fig. 4 and detailed list below).

We investigated asymmetry-cognition relationships using 2-tailed Pearson coefficients for correlations between caudate asymmetry index and age-adjusted CVLT-C and DAS-2 measures.

CVLT-C:	discriminability	(r^2=0.13, p<0.02);
	long delayed cured recall	(r^2=0.11, p<0.03)
	short delay cued recall	(r^2=0.13, p<0.03);
DAS-2:	general cognitive ability (GCA)	(r^2=0.10, p<0.04);
	spatial ability	(r^2=0.09, p<0.05);
	sequential & quantitative reasoning ability	(r^2=0.11, p<0.03);
	verbal similarities ability	(r^2=0.13, p<0.02).

No such correlations existed in the control population for any of these measures.

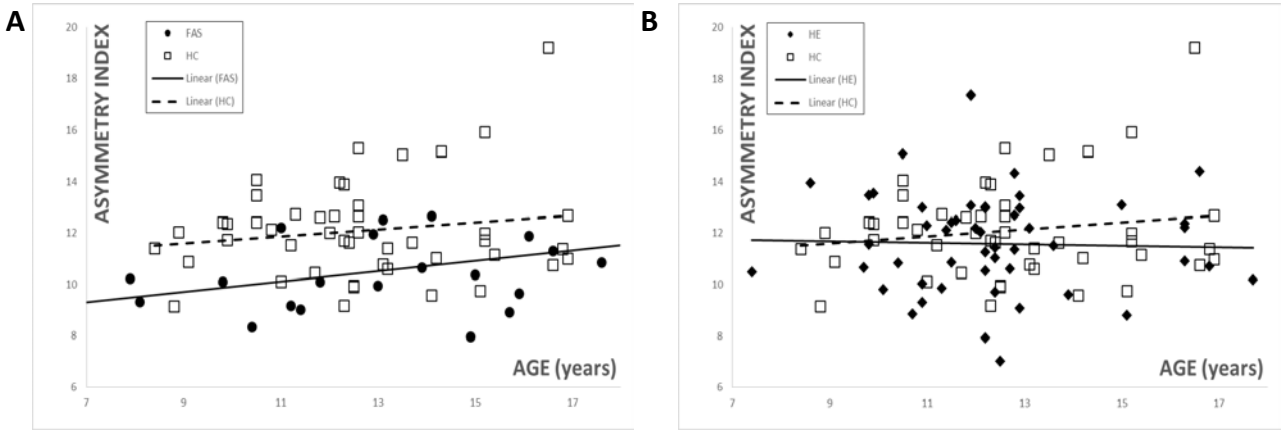


Figure 4 Caudate nucleus asymmetry index (distance between DSM representations of caudate nucleus and its reflection) against age for A) FAS vs Control and B) HE vs Control. The control group has a significantly higher mean asymmetry index (12.1) compared to that (10.5) of the FAS group (p<0.002). In contrast, there is no significant difference between controls and HE.

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

FETAL BRAIN STRUCTURE IDENTIFICATION IN OXFORD

It was not possible to fill the designated postdoctoral vacancy until 2018. The nominated appointee, Ruobing Huang, submitted her Oxford DPhil thesis on January 12th and has now started work on the project supervised by Professor Alison Noble. Her thesis work has been focused on standard foetal neurosonography examinations for evaluating key brain structures (Fig. 5a) from their identification and segmentation within ultrasonographs (Fig. 5b).

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.

Figure 5 Identification of fetal neural structures in ultrasonographs

NEONATAL FACE ANALYSIS IN BRIGHTON

The subcontracted collaboration with Dr Neil Aiton on neonatal facial analysis has been considerably delayed by contract negotiations between the University of Oxford and Dr Aiton's Health Trust as well as the unexpected requirement to obtain ethics approval for the 3D facial analysis (despite already having ethics approval for 2D imaging). A Canfield H1 3D camera and supporting laptop has been acquired by Oxford for use in Brighton. In order to address the rather short 4-month period remaining in the current CIFASD4 first year programme, Dr Aiton has arranged to double the previously planned weekly commitment.

Neil Aiton and research midwife, Anna Ferguson, attended a training session in Oxford in using the camera and the image acquisition software provided by the camera manufacturer. They also met with Alison Noble and Ruobing Huang to discuss protocols for prenatal ultrasound and neonatal photographic imaging as well the potential for neonatal imaging of transfontanelle ultrasound imaging of brain structures.

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?

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).

Mike Suttie has been a registered part-time doctoral student at UCL throughout his involvement in CIFASD. He will submit his PhD thesis at the end of February 2018. It will be examined in late March 2018.

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:

3D Face Analysis Popup Workshop, Oxford

Peter Hammond organized a pop-up 3D Face Analysis Workshop for 25 attendees at the (Li Ka Shing) Big Data Institute in Oxford at which he and Mike Suttie presented material on FASD face analysis and related technical issues. Dr Peter Claes was also one of the speakers and this led to his invitation to attend the CIFASD February 2018 face-to-face meeting.

The Li Ka Shing Foundation annual East West Alliance meeting, Oxford

This international symposium of about 200 participants is a showcase of research involving computer-based analysis of biomedical and health care data that is undertaken by universities and institutes funded by the Li Ka Shing Foundation (who funded most of the Big Data Institute construction). A conventional poster was supplemented by live demonstrations of 3D face image capture and automated analysis using the Bellus 3D camera and our face screening software.

Meeting of the UK House of Commons All Parliamentary Party Group (APPG) on FASD, London

Mike Suttie and Peter Hammond were invited to attend this APPG on FASD by the Secretariat that includes Julia Brown one of the founders of the UK FASD Trust. Peter Hammond was one of four expert witnesses presenting FASD related material to UK members of parliament. Dr Neil Aiton, one of the new CIFASD participants, was also in attendance.

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

For the four-month period of February to May 2018, we plan to do the following:

- complete a more formal evaluation of the potential use of the face screening tool in the FASD clinic
- continue to develop 2D face analysis using methods and software developed for 3D
- provide Oxford colleague Chris Nellaker with access to the artificially generated 2D images for testing customised 2D facial analysis software for control-FASD discrimination
- recruit and capture 3D photos of newborn infants in 3 categories: no and low alcohol exposure groups (from postnatal ward), significant alcohol exposure (from specialist clinic)
- test Ruobing Huang's brain segmentation algorithms on CIFASD ultrasound images
- renew collaborative links with Professor Hein Odendaal in South Africa with a view to obtaining access to more of the ultrasound images collected within the NIAAA-funded PASS consortium.

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

NIH Public Access Compliance	Citation
PMC Journal In Process	Dou X, Menkari C, Mitsuyama R, Foroud T, Wetherill L, Hammond P, Suttie M, Chen X, Chen SY, Charness ME; and the Collaborative Initiative on Fetal Alcohol Spectrum Disorders.. L1 coupling to ankyrin and the spectrin-actin cytoskeleton modulates ethanol inhibition of L1 adhesion and ethanol teratogenesis. FASEB J. 2017 Nov 6. pii: fj.201700970. doi: 10.1096/fj.201700970. [Epub ahead of print] PubMed PMID: 29109170. [Epub ahead of print]
Complete	Fish EW, Wieczorek LA, Rumple A, Suttie M, Moy SS, Hammond P, Parnell SE. The enduring impact of neurulation stage alcohol exposure: A combined behavioral and structural neuroimaging study in adult male and female C57BL/6J mice. Behav Brain Res. 2018 Feb 15;338:173-184. doi: 10.1016/j.bbr.2017.10.020. Epub 2017 Oct 28. PubMed PMID: 29107713; PubMed Central PMCID: PMC5726510.

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

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, submitted to ACER.

Aiton NR, Gourlay E, Monk V, Thorup K. Using Facial Photography and Computerised Facial Analysis to Determine Facial Characteristics Associated with Prenatal Exposure in Newborn Infants. (in preparation).

***Poster Abstracts and Presentations**

Li Ka Shing Symposium, Big Data Institute, Oxford.

UK APPG on FASD Houses of Parliament, London.

3D Face Analysis Popup Workshop, Oxford.

Hammond P, Suttie M, Muenke M, Kruszka P, Adeyemo A. Detecting & predicting syndromic phenotypes in diverse populations using facial proxies & normative cohorts. 2nd Big Data in Biology and Health Conference, Wellcome Genome Campus, Cambridge, UK.

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

Jeff Wozniak (Minnesota) – major interaction on production of paper on face-brain-cognition correlates

Tatiana Foroud (Indiana), Clare Coles (Emory) and Joanne Weinberg (Calgary) – production of protocol for 2D image capture and assistance with ethics proposals for 2D image acquisition

Scott Parnell (North Carolina) – contribution to further mouse brain studies and resulting publication.

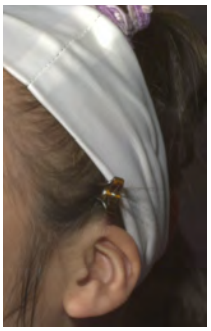
APPENDIX CIFASD4 PROTOCOL FOR CAPTURE OF 2D FACIAL PHOTOGRAPHS

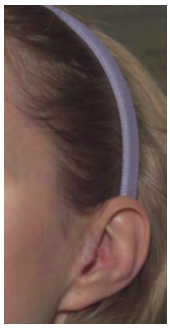
These notes and protocol are designed to optimise the capture of 2D facial photographs. Initially, 2D images will be imported into 3D models of face shape so should be at least as well taken as the 3D.

Camera operators It pays to have as few camera operators as possible. Maintenance of standards of image capture benefits from regular camera use. Large gaps between operation diminishes efficacy or causes lapses in adherence to the protocol. Requests to keep hair away from the face, especially the ears, can be forgotten and spoil subsequent analysis.

Preparing the camera setting when undertaking a large series of images An office type chair on wheels with vertical seat adjustment (preferably gas powered) and back rest adjustment really helps. Remember it is easier to set the chair high and use weight to adjust downwards. If possible use a camera fitted to a tripod to keep its position constant while adjusting the subject e.g. rotating the chair gently to get the profile views while maintaining head posture. It is useful to have available small toys, puppets, hair bands/grips/clips/slides, bells, tissues, face wipes etc. Occasionally, subjects will not or cannot be photographed out of a stroller or mobility chair. Anticipate this by allowing sufficient room for it to be manoeuvred into place. A well-lit area with a **PLAIN WHITE BACKGROUND** (or at least monochrome) is preferable to assist subsequent analysis.

Preparing the subject If there are many individuals awaiting imaging, have someone separately consent them and ensure that hair and clothing do not occlude the face. Glasses **MUST** be removed. Ear rings and piercings, especially in the lower ear lobe, are best removed as the lower ear attachment is an important landmark to capture. The **MOST** important aspect of preparing the subject is to ensure that hair is pulled away from the face and ears as much as possible.

A 

B 

Hair bands are useful, even for male subjects. Long hair can be pulled into a pony tail and held with “scrunchies” and the like. Small hair clips/grips are easier to manipulate and more hygienic –parents can be worried about hair lice! Consider hair gel, often accepted by teenagers, or wet hair – to flatten thick side burns. Thick hair pushed behind ears can result in women’s ears appearing more prominent than they are! Better to use hair band, scrunchy or grips/pins.

Posing and gaining the attention of the subject For children unable to follow instructions to pose, a small toy can be held in the operator’s free hand centrally and close to the camera, slowly lifted to engage the child’s gaze and then when the face is judged (with practice) to be at an appropriate angle, the picture can be taken. For babies and very young children, use a bright light source or even a small bell to attract attention. With children, it is best initially to see what pose they adopt naturally. Some will recall school photos and grin broadly. Others will close their mouth without encouragement. The danger is that if you ask some children to close their mouths and place lips together, they will clench their teeth and tense their lips in an unnatural fashion. Very young children, or those who are difficult to control, may have to perch on a parent’s lap or knee. It is important then to ensure that clothing doesn’t runkle up and occlude the lower parts of the face. It is best if the parent tries to keep their own face out of view – although they can be edited out.

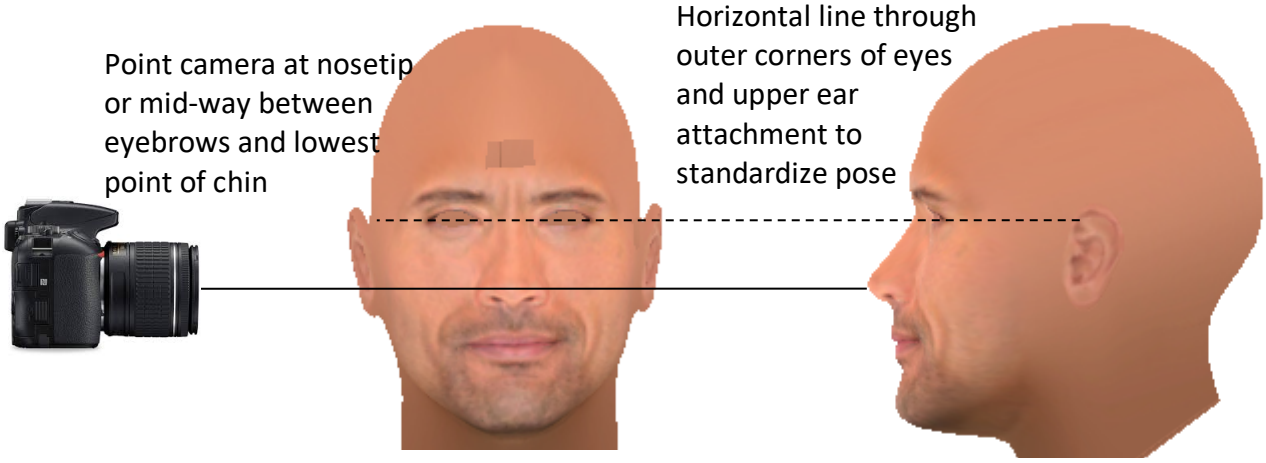
PROTOCOL FOR 2D IMAGE CAPTURE

SUBJECT PREPARATION AND POSE

Draw hair back from forehead and ears as much as possible
Remove glasses, ear rings/piercings and encourage subject to keep eyes open
Discourage smiling and try for a neutral expression
Ask for lips to be gently closed together (if dental malocclusion forces lips apart discourage their being forced together as this will distort the philtrum and lip shape)
Take a single portrait without adhesive labels
Position adhesive labels on forehead above brow ridge and on left cheek as below to take 3 additional images with labels

PORTRAIT **THREE-QUARTER PROFILE** **PROFILE**

CAMERA POSITION RELATIVE TO SUBJECT



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

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?

The specific objectives for the current funding year and progress made toward those objectives are listed in **Figure 1.**

Figure 1. Progress made toward specific objectives for funding year 1.

January 2018

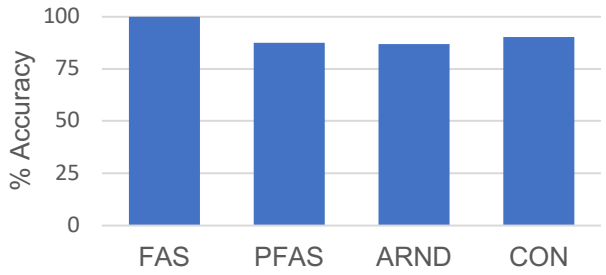
Mattson Timeline for Year 1 (July-May)

	Aim 1a (Archival Data)	Aim 1b (eTree)	Aim 1c/2b (Validation)	Aim 2a (FONS)
Collaborators	Chambers	Jones, Wozniak, Admin Core	Jones, Wozniak, Foroud	Foroud, Hammond
Interactions	In email contact, reviewing data dictionaries (coFASP/CIFASD)	In touch via in-person and phone meetings	In touch via in-person and phone meetings. Have regularly scheduled monthly call, need to schedule additional calls	✓ Monthly conference calls with with TF and SM
July-August	✓ IRB Approval ✓ Request Data (in process)	✓ IRB Approval ✓ Hiring	✓ Hiring ✓ Purchasing NP Materials	✓ FONS Planning ☐ FONS Development
September	✓ Obtain Data ✓ Begin Data Analysis	✓ Training ✓ eTree Development ✓ Programming (Admin Core) ✓ Establish subcontract ✓ Practice Clinic 9/13/17	✓ Training on Battery (local) ✓ Training on Battery (UMN) Scheduled 9/8-9 ✓ Transition to electronic data collection	☐ FONS Programming (Admin Core) ☐ Piloting
October-December	✓ Data Analysis ✓ Preliminary Results	☐ Make adjustments to eTree App ☐ Data Collection in San Diego	✓ Pilot Testing	
Jan-March	✓ Refine Tree, Rerun Analyses (if needed)	☐ Data Collection in Minnesota		
April - May (June)	☐ Present Results at RSA ☐ Prepare Manuscript		☐ Data Collection	

The major activities and relevant results related to these objectives are as follows:

Archival Data Analysis (Aim 1a). We requested and obtained archival data from the San Diego site of the CoFASP project from Dr. Chambers. We applied the decision tree to this data set. Data from 814 children aged 5-7y (*M*=6.8) were selected for analysis: 64 children who received an alcohol-related study diagnosis (FAS, pFAS, or ARND based on Hoyme et al., 2016) and had reported prenatal alcohol exposure and 750 children, who had neither reported histories of prenatal alcohol exposure nor an alcohol-related diagnosis. The original decision tree correctly classified 71.7% of the sample using the first entry point (behavior ratings first) and 50.8% of the sample using the second entry point (dysmorphology variables first). To improve accuracy, criteria were altered and the tree was retested. With revised criteria, the decision tree correctly classified 100% of subjects with FAS, 87.5% of subjects with pFAS, 87.0% of subjects with ARND, and 90.9% of controls (see **Figure 2**). Overall, the revised decision tree accurately classified 90.4% of the sample. These results support the use of the decision tree in both high- and low-risk samples. We submitted an abstract describing this study to RSA and hope to present it at the conference in June 2018. In addition, we plan to re-apply the revised tree to our existing CIFASD 2/3 sample to test its accuracy in our clinical comparison group. Additional revisions to the tree will be made if necessary. We will also test our revised model in the other CoFASP site (PI May) and Dr. Chambers Ukraine sample.

Figure 2: Results of Revised Decision Tree Applied to Low-Risk Archival Data (CoFASP San Diego)



eTree (Aim 1b). During the current funding year, we developed a prototype mobile application (see Figure 3) for the Decision Tree (the eTree) in conjunction with Dr. Ganz Chockalingam, a developer supported by the administrative core. The eTree app is illustrated in Figure 3. We have started using the app with patients referred by Dr. Jones through his FASD/Dysmorphology Clinic. The number of patients referred, consented, and enrolled is listed in Table 1. Based on the data we have collected so far, we have several proposed alterations for the eTree. We are currently working with Dr. Chockalingam to implement these changes.

Figure 3. Illustration of eTree App on iPad home screen (left) and layout of eTree screens within the app (right).

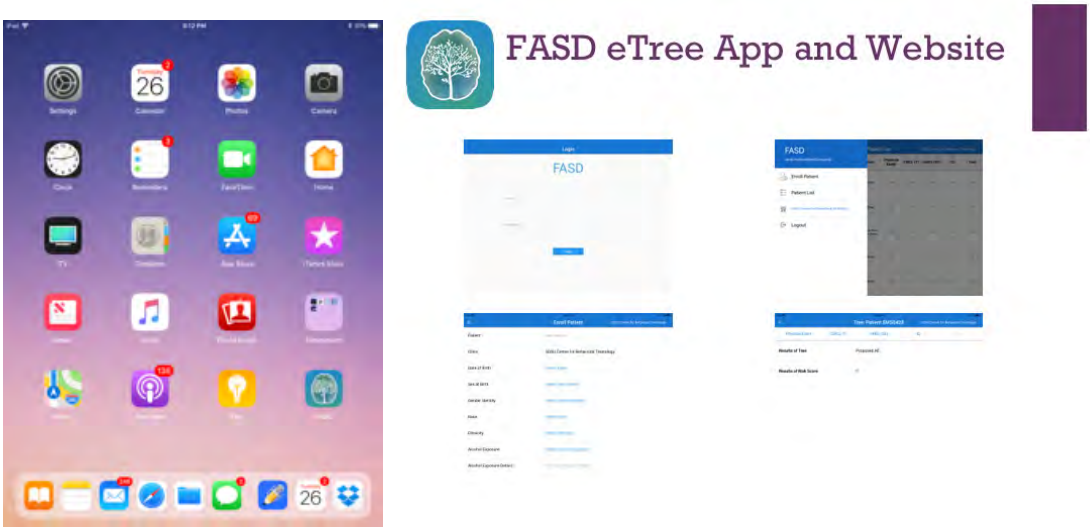


Table 1. Number of Subjects Recruited and Enrolled in Tree Study (Aims 1b/1c)

Site	Agreed to Contact	# System Email	#Consented	#Complete/Entered	#Scheduled for NP Validation
UCSD/FASD	35	33	15	15	2
SDSU/CBT	10	10	10	10	1
Total	45	43	25	25	3
Number proposed (Y1)	40				5

Validation Test Battery (Aims 1c/2b). We proposed a neuropsychological test battery to validate the eTree results. During the current funding period we finalized our test battery, purchase materials, transitioned all testing to electronic platforms, modified our IRB, trained personnel and piloted testing procedures. We proposed to start conducting the validation test battery in month 10 of the first year (March or May, depending on when you start year 1). We have just scheduled our first 3 validation subjects for testing. Dr. Wozniak is also administering this validation test battery in Minneapolis and has tested 4 subjects.

FONS/Web Portal (Aim 2a). The final aim of the proposal is to develop and implement a novel online neurobehavioral screening tool to be integrated into the WebPortal as proposed by Dr. Foroud. We have regularly scheduled conference calls to discuss this developing project. In San Diego, we have reviewed the literature on electronic testing of cognitive and behavioral functioning and are planning the test battery. We will develop the FONS with the assistance of Dr. Dean Delis, a consultant on the project. The same in-person test battery will be used to validate the online results (Aim 2c).

B.3 Competitive Revisions/Administrative Supplement. 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 reporting period, we plan to continue our efforts in meeting all our aims. As proposed, we will complete the analyses and prepare the paper for Aim 1a (archival data analysis), revise the eTree to reflect the results of the archival data analysis, and continue to recruit subjects from the dysmorphology and CBT clinics (Aim 1b). We will conduct validating neuropsychological testing on a subset of the eTree subjects (Aim 1c). In conjunction with Dr. Wozniak, we will evaluate subjects at the Minneapolis site using the eTree and validation test battery. We will develop and pilot the online testing procedures for implementation in the WebPortal (Aim 2a).

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

NIH Public Access Compliance	Citation
Complete	Infante MA, Moore EM, Bischoff-Grethe A, Tapert SF, Mattson SN, Riley EP. Altered functional connectivity during spatial working memory in children with heavy prenatal alcohol exposure. Alcohol. 2017 Nov;64:11-21. doi: 10.1016/j.alcohol.2017.05.002. Epub 2017 Aug 12. PubMed PMID: 28965651; PubMed Central PMCID: PMC5635832.
Complete	Glass L, Mattson SN. Fetal Alcohol Spectrum Disorders: A Case Study. J Pediatr Neuropsychol. 2017 Jun;3(2):114-135. doi: 10.1007/s40817-016-0027-7. Epub 2017 Jan 30. PubMed PMID: 28948136; PubMed Central PMCID: PMC5609722.
Complete	Gross LA, Moore EM, Wozniak JR, Coles CD, Kable JA, Sowell ER, Jones KL, Riley EP, Mattson SN; CIFASD.. Neural correlates of verbal memory in youth with heavy prenatal alcohol exposure. Brain Imaging Behav. 2017 Jun 27. doi: 10.1007/s11682-017-9739-2. [Epub ahead of print] PubMed PMID: 28656347; PubMed Central PMCID: PMC5745322. [Epub ahead of print]
Complete	Wozniak JR, Mueller BA, Mattson SN, Coles CD, Kable JA, Jones KL, Boys CJ, Lim KO, Riley EP, Sowell ER; CIFASD.. Functional connectivity abnormalities and associated cognitive deficits in fetal alcohol Spectrum disorders (FASD). Brain Imaging Behav. 2017 Oct;11(5):1432-1445. doi: 10.1007/s11682-016-9624-4. PubMed PMID: 27734306; PubMed Central PMCID: PMC5389933.

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

Wozniak, J.R., Boys, C. Hendrickson, T., Mueller, B.A., Jones, K.L., Riley, E.P., Lim, K.O., Kable, J.A., Mattson, S N., Coles, C.D., Sowell, E.R. Two-year cortical trajectories are abnormal in children and adolescents with prenatal alcohol exposure. Submitted August, 2017. (Concept Proposal #7).

Uban, K.A., Kan, E., Wozniak, J., Mattson, S., Coles, C.D., Sowell, E.R., & the CIFASD. The relationship between socioeconomic status and brain development is attenuated in children and adolescents with prenatal alcohol exposure. Submitted July 2017. (Concept Proposal #34 or 35?).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. Cognitive predictors of communication impairment in youth with histories of heavy prenatal alcohol exposure. Submitted May 2017. (Concept Proposal #47).

*** Poster Abstracts and 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. To be presented at the Research Society on Alcoholism meeting, San Diego, June 2018.

Inkelis, S.M., Moore, E.M., Mattson, S.N., Riley, E.P. (2018). Predicting prenatal alcohol exposure histories using measures of attention and activity. To be presented at the Research Society on Alcoholism meeting, San Diego, June 2018.

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. To be presented at the Research Society on Alcoholism meeting, San Diego, June 2018.

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. To be presented at the Research Society on Alcoholism meeting, San Diego, June 2018.

Cheney, D.C., 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. (2017). Development and validation of a risk score that correlates with neurobehavioral effects of prenatal alcohol exposure. Presented at the Research Society on Alcoholism meeting, Denver, June 2017.

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. (2017). Relation between adaptive function and IQ among youth with histories of heavy prenatal alcohol exposure. Presented at the Research Society on Alcoholism meeting, Denver, June 2017.

Infante, M.A., Moore, E.M., Bischoff-Grethe, A., Tapert, S.F., Mattson, S.N., and Riley, E.P. (2017). Spatial working memory in children with heavy prenatal alcohol exposure: A multimodal imaging approach. Presented at the Research Society on Alcoholism meeting, Denver, June 2017.

Inkelis, S.M., Thomas, J.D., Coles, C.D., Kable, J.A., Wozniak, J.R., Riley, E.P., Mattson, S.N., and the CIFASD (2017). The relationship between sleep problems and behavioral functioning in 5-year-olds with prenatal alcohol exposure. Presented at the Research Society on Alcoholism meeting, Denver, June 2017.

Moore, E.M., Haven, S., Gonzalez, G., Murphy, C., Jones, K.L., Mattson, S.N., Giedd, J., and Riley, E.P. (2017). Impaired olfactory threshold detection in children with FASD. Presented at the Research Society on Alcoholism meeting, Denver, June 2017.

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 funded project is to improve identification of individuals affected by prenatal alcohol exposure, which is consistent with the primary goals of the CIFASD consortium. The involves direct collaborations with other U01 projects including those of Drs. Foroud (Aim 2), Hammond (Aim 2), Chambers (Aim 1), and Wozniak (Aims 1 and 2). Aim 1 is conducted in close collaboration with Dr. Chambers (analysis of archival data) and Dr. Jones (recruitment/referral/evaluation of subjects). The eTree app is being developed with additional support from the Administrative Resource. The current project also interacts with Drs. Petrenko and Weinberg (dissemination of information about relevant projects) will Dr. Wozniak (administration of eTree and validation neuropsychologic test battery).

If a project you were the PI on was part of Phase III of CIFASD, use this space to update the Advisory Committee on any new progress not previously reported.

See above text.

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?

1) Major Activities. Aim 1: To date we have utilized RNA-Seq to explore the baseline differences 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 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, providing a complete picture of gene expression in each strain of mouse. 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.

Aim 2: We have raised a stock of mutagenized zebrafish with which to perform our analyses. These fish will be crossed to a stock of *pdgfra* heterozygotes to identify modifiers of ethanol teratogenicity on this genotype. These crosses will result in 25% of embryos carrying a mutation in both *pdgfra* and a potential modifier gene. Due to the fecundity of zebrafish, we will typically obtain more embryos then

necessary for the suppressor screen that was proposed. Therefore, we will also seek to identify enhancer mutations to more thoroughly dissect the pathway regulating the ethanol sensitivity of *pdgfra* heterozygotes.

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: Our initial analyses of our first set of experiments has revealed that after correction for multiple comparisons, the baseline differences between 6J's and 6N's included over 80 differentially regulated genes and over 100 altered gene networks. Among the differentially regulated genes were several genes involved in primary cilia formation and function, genes important in G-protein signaling, numerous genes regulating inflammatory homeostasis, and genes in the PI3k/AKT/mTOR pathway. Importantly, many of these genes and pathways that are significantly altered in 6J's compared to 6N's at baseline regulate the balance between normal cell cycle progression and apoptotic death. For example, increased expression of immune pathways at baseline, such as those related to neuroinflammation and cytokine expression (e.g. IL-6 signaling), as well as down-regulation of the PI3k/AKT pathway, could prime embryonic cells to be more likely to undergo apoptosis in response to ethanol, resulting in increased cell death and more severe craniofacial anomalies.

Aim 2: We have performed a dose response curve in our *pdgfra* carrier line to identify 1) an ethanol dose that is just below the level of effect on *pdgfra* heterozygotes and 2) a dose that maximally effects heterozygotes. These exposed zebrafish are currently being genotyped.

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. A mutant in an additional gene of interest is already available. 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.

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?

Dr. Karen Boschen, a second-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 will begin in March 2018.

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

The results of our first analyses have been shared with the Human Genetics component (Foroud) of CIFASD. In addition, we have submitted an abstract for the annual RSA meeting in June 2018.

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

Aim 1: During the next period, we will use zebrafish and CRISPR/Cas9 to further refine our candidate gene list obtained from our 6J/6N baseline RNA-Seq data. We will also perform and analyze further RNA-Seq experiments in mice to explore in an unbiased manner these two strains' initial response to an acute alcohol exposure. Aim 2: Our modifier screen will be well underway and any genes identified in the human genetics analyses (Foroud) will be prioritized for analyses in zebrafish. Based on results from previous research and confirmations from the first Aim 1 experiments, we will be exploring the role of p53 in modifying susceptibility to ethanol in the mouse.

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

NIH Public Access Compliance	Citation
Complete	Fish EW, Murdaugh LB, Sulik KK, Williams KP, Parnell SE. Genetic vulnerabilities to prenatal alcohol exposure: Limb defects in sonic hedgehog and GLI2 heterozygous mice. Birth Defects Res. 2017 Jul 3;109(11):860-865. doi: 10.1002/bdr2.1026. Epub 2017 May 15. PubMed PMID: 28504423; PubMed Central PMCID: PMC5495621.
Complete	Fish EW, Wieczorek LA, Rumple A, Suttie M, Moy SS, Hammond P, Parnell SE. The enduring impact of neurulation stage alcohol exposure: A combined behavioral and structural neuroimaging study in adult male and female C57BL/6J mice. Behav Brain Res. 2018 Feb 15;338:173-184. doi: 10.1016/j.bbr.2017.10.020. Epub 2017 Oct 28. PubMed PMID: 29107713; PubMed Central PMCID: PMC5726510.

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

Fish E.W., Murdaugh L.B., Boschen K.E., Mendoza-Romero H.N., Tarpley M., Chdid L., Zhang C., Boa-Amponsem O., Mukhopadhyay S., Cole G.J., Williams K.P. and Parnell S.E. Cannabinoids exacerbate alcohol teratogenesis by disrupting Sonic Hedgehog signaling through a novel interaction between the CB1 receptor and Smoothened. In preparation. (Concept Proposal #21.)

Mendoza-Romero H.N., Baker L.B. and Parnell S.E. The pro-apoptotic protein Bax modifies susceptibility to early gestational alcohol exposure. In preparation. (Concept Proposal #46.)

*** Poster Abstracts and Presentations**

Boschen K.E., Eberhart J.K. and Parnell S.E. Transcriptome-wide analysis of ethanol sensitive and insensitive mouse strains during early embryonic development. Submitted for presentation at the 2018 RSA meeting.

Boschen K.E. and Parnell S.E. Transcriptome-wide analysis in the neural tube following mid neurulation stage ethanol exposure in C57BL/6J mice. Submitted for presentation at the 2018 RSA meeting.

Boschen K.E., Fish E.W. and Parnell S.E. Prenatal ethanol exposure induces a “transient ciliopathy”: A novel mechanism for ethanol’s pathogenesis. Submitted for presentation at the 2018 RSA meeting.

Fish E.W., Boschen K.E., Leitzinger J.O., Venktatasubramanian D. and Parnell S.E. The primary cilia gene *Kif3a* mediates vulnerability to the effects of neurulation stage alcohol exposure on adolescent exploratory behavior. Submitted for presentation at the 2018 RSA meeting.

Describe your project’s interrelation with aims of the CIFASD consortium its other projects and the progress on the interactions. We have sent a preliminary, informal report to the Human Genetics component of our initial candidate gene list that the Eberhart Lab will further confirm in the fish model, then the Human Genetics component can *a priori* examine those genes in their whole exome sequencing data.

If a project you were the PI on was part of Phase III of CIFASD, use this space to update the Advisory Committee on any new progress not previously reported. We submitted RSA abstracts on the primary cilia data, and will soon begin manuscript preparation. We are nearly completely finished with the alcohol/cannabinoid manuscript and will submit that soon.

Principal Investigators: Christie L. M. Petrenko and Cristiano Tapparello
Institutions: University of Rochester, Seattle Children's Research Institute (Subcontract)
CIFASD4 Project Title: Development and Evaluation of an Evidence-Based Mobile Health Caregiver Intervention for FASD
Grant Number: U01AA026104

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?

1) Major Activities. Over the first six months of the project, we have accomplished the following activities:

- Hired and trained relevant staff; established responsibilities and team communication strategies.
- Refined app components and functionalities to be developed (see figure 1 for illustration).
- Set up the app framework on iOS for development and testing. Consulted with Dr. Chockalingam, as facilitated by the Administration Core.
- Drafted all 12 core Learning Modules (> 100pgs) based on the standard Families Moving Forward (FMF) Program content, principles, and methods, and added unique content and features. Completed first round of intense editing (>64hrs). Optional content (~40% complete) and revisions of core content (~ 80% complete) are in progress.
- Interface design mockup completed and organized in an interactive prototype. Graphical interface programmed into iOS (CIFASD major milestone #1 attained on schedule).
- Initial Family Forum functionalities have been programmed in both iOS and Android. Cross-platform testing is in progress.
- 2 focus groups have been completed in Rochester, NY (preliminary themes presented below). Logistical planning is underway for additional focus groups in Rochester, Washington DC, Atlanta, Minneapolis, and San Diego with the assistance of other CIFASD investigators.
- Interviews with multiple videographers were completed in January and videographer has been selected. Contract is in progress. Production will begin in March.
- We are making excellent progress in app development and are on course to complete all planned activities on schedule.

2) Specific Objectives. 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.

We are assessing attainment of these objectives in two primary ways. The first is producing the app on schedule based on pre-determined internal team benchmarks and major milestones reported to CIFASD. As detailed above, we are making good progress in developing the app and are on or ahead of schedule for all benchmarks and milestones. We have a cohesive and collaborative multidisciplinary team that works efficiently to develop the content and technological aspects in a coordinated fashion.

Our second assessment method is using systematic qualitative methods to collect data from parents and caregivers of children with FASD (key stakeholders) to evaluate how well the app we are developing meets the needs of families on important outcomes. During the initial development phase, we are using focus group methods to solicit the perspectives from diverse families on aspects such as the interface design, ease of use, relevance of components and content, and barriers and facilitators of use. Following the completion of the initial prototype, we will have a small number of families try out the app for a period of time and complete individual interviews with them regarding their experience with the app. Qualitative findings will inform refinements in the app prior to larger scale feasibility and effectiveness trials in aims 2 and 3. Preliminary findings from our first round of focus groups completed in December 2017 are summarized below.

Aim 1 is to be 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. Within CIFASD we have established the following major milestones for the Development Phase (Years 1 & 2):

FMF Connect App Development Major Milestones	Goal Date
Design interface programmed	1/31/18
Family Forum programmed	5/31/18
Psychoeducational content written & programmed	7/31/18
Audiovisuals produced & programmed	9/30/18
Initial Feasibility Prototype complete	2/28/19

Once the initial feasibility prototype is completed, we will conduct an initial test with 5 participants, to be completed by 5/31/19. As described in B.2, we are making excellent progress on Aim 1 and are on track to complete all project goals as planned.

3) Significant Results. To date, we have completed two initial focus groups in Rochester, NY including a total of 8 parents and caregivers. Several preliminary themes emerged during focus group discussions:

First, participants felt strongly that users should be able to use the app indefinitely and be able to return to helpful content and maintain engagement with the Family Forum, even after completing all of the learning modules (themes: **access, sustained engagement**). The theme of **personalization** was also highlighted. They liked that users could save content of interest into the Notebook for later reference. They were also positive about the Tools in the Notebook and suggested additional tools (e.g., medication tracker) for consideration.

The Family Forum was a major topic of conversation in focus groups. Participants emphasized the themes of **trust, confidentiality, and protections** for this component. Many participants have participated in other forums or online groups and shared their experiences of things that made them more or less likely to participate. Having peer moderator(s) in the Family Forum was positively received, and additional protections and functionalities were recommended. Participants suggested access to specific sub-forums be "unlocked" as users complete relevant learning modules. This would also limit the risk of people downloading and viewing/posting in the Forum inappropriately. Participants also liked the idea of having sub-forums available based on geographic location to facilitate sharing resources and advocacy tips.

Participants had a strong, positive reaction to the Learning Module interface design. They identified the design conveyed **hope, well-being, and happiness**. Some felt the face on the sun might convey the app is for children or is too "**child-like**" and discussed some alternatives. The logo suite received less enthusiasm, but generally elicited neutral to positive comments. Participants preferred graphics to photos in the interface design.

Facilitators and barriers to use were also discussed, both in terms of content/features and technology. Participants identified primary **motivators** for app usage as 1) when the child is struggling (i.e., parents looking for answers) and 2) connections with other users in the Family Forum. Technology features that would facilitate use would be voice-to-text functionalities (i.e., to reduce need to type in responses or posts) and the ability to transition across platforms (e.g., phone, computer) to access the app. **Barriers** to use included a poorly moderated Family Forum or other concerns of trust and safety. Participants identified they would be less likely to use the app if it was difficult to navigate, was slow to load, or required lots of scrolling.

4) Key Outcomes and Other Achievements. We will continue the iterative process of developing the app, soliciting input from key stakeholders, and refining functionalities over the course of the next 18 months, as outlined in B.6. This will result in a rigorously designed app that has high potential for success in meeting family needs and improving outcomes, as 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.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?

Project team members have actively sought out additional training and professional development to enhance their completion of activities supported by the project. Jennifer Parr, the research coordinator on the project, 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. Parr's contributions to content development and team discussions on app functionalities.

Drs. Petrenko and Carmichael Olson attended the 4th Biennial Society for Implementation Research Collaboration (SiRC) Conference focusing on "Mechanisms of Implementation: What Works and Why?" from September 8-9, 2017. There they learned about recent trends in implementation science research. A plenary on the Behavior Change Techniques Taxonomy developed by Dr. Susan Michie

and her team was particularly influential in our process of refining the app functionalities and content design.

We also sought consultation from Dr. Eric Frederiksen, an expert in online learning and instructional design at the University of Rochester Warner School of Education in September, 2017. He conducted a training for us on "backward design," which is a rigorous process used in instructional planning to achieve specific learning goals (outcomes). Drs. Petrenko, Tapparello, and Carmichael Olson and the project coordinator Jennifer Parr attended this training. These same team members also consulted with an expert in ethics of technology use in interventions, Dr. Anthony Pisani, who also serves as a member of our Independent Safety Monitoring Committee. These consultations and trainings were quite useful as we developed core content for the app and considered refinements for app functionalities.

Dr. Tapparello refined his knowledge in app design and iOS programming through individual study of professional tools for app wireframing and prototyping (like, e.g., Sketch and inVision) and the iOS human interface guidelines. Rebecca Van Dyke, the research assistant working on the app development, received one-on-one training with Dr. Tapparello on different techniques and common practices used in the development of cloud-based mobile applications. This training has facilitated Ms. Van Dyke's contributions to the implementation of the Family Forum functionalities.

B.5 How have the results been disseminated to communities of interest? None to date.

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

Over the next six months, we intend to do the following:

- Complete the first draft of optional Learning Module content and begin first round of edits of optional content. Complete revisions of core content.
- Begin programming Learning Modules.
- Complete programming of the Family Forum (CIFASD major benchmark #2).
- Conduct focus groups in Washington DC, Atlanta, Minneapolis, and another round in Rochester. (San Diego focus groups targeted to coincide with RSA in June).
- Produce videos for Learning Modules.

These plans are consistent with what was proposed in the grant application, with one minor exception. We initially specified conducting focus groups in Rochester, NY. We have subsequently decided to expand qualitative data collection to other regions in the US, given expected regional variability in resources and services available to families. Our total sample size for this component will increase as a result. We believe this expansion will enhance the rigor of our qualitative analyses in Aim 1 since it will allow us to elicit perspectives of participants that more comprehensively resemble the target population for this app (parents/caregivers raising children with FASD in the US). We have been able to connect with other CIFASD researchers and partners (i.e., Coles, Wozniak, and Riley, and Kathy Mitchell of NOFAS) to begin facilitating setting up the logistics for these focus groups.

***Publications** **[Accepted & In Press]** None to date.

***Publications** **[In Preparation & Submitted]** None to date.

***Poster Abstracts and Presentations** None submitted to date. We are considering submissions for a couple of future conferences.

Describe your project's interrelation with aims of the CIFASD consortium, its other projects, and the progress on the interactions. The purpose of the CIFASD consortium is to inform and develop effective interventions and treatments for FASD through multidisciplinary research. Overarching aims of projects in CIFASD-IV include better identification of people prenatally exposed to alcohol, elucidation of factors that increase or reduce risk to FASD, understand the effects of prenatal alcohol exposure across the lifespan, and develop treatments for FASD.

Our project fits squarely within the mission of CIFASD, with its overall objective to develop and evaluate an accessible and scalable mobile health intervention for families that has the potential to surmount some existing barriers to care. We have planned interactions with other projects through facilitation of recruitment into our projects (e.g., projects lead by Foroud, Mattson, Wozniak, Coles, Jones). As mentioned above, we have been in contact with Drs. Wozniak, Coles, and Riley regarding setting up focus groups in their respective cities this spring and summer. In future years, we also will consult with Dr. Jones within the Dysmorphology core for trials in later aims. Our project will provide data for inclusion in the data repository and content for dissemination to broader communities of interest.

Principal Investigator: Joanne Weinberg
Institution: University of British Columbia
CIFASD4 Project Title: Immune Dysregulation in FASD: Programming of Health and Neurobehavioral Outcomes
Grant Number: U01AA026101

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

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 U01 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 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) Wozniak: new *child cohort* recruited for choline studies.

Data from this work will increase our understanding of immune variables as biomarkers of alcohol intake and 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?

1) Major Activities. As detailed in our Specific Aims, our grant has two Aims, reflecting two different areas of focus. The focus for Aim 1 is on pregnant women, infants and children, while the focus for Aim 2 is on adults with FASD. For the Adult Study we are collaborating closely with two groups: 1) Dr. Tim Oberlander and his team at BC Children's Hospital who have extensive clinical and research experience with children with FASD, as well as a state-of-the-art Biobehavioral Testing Facility with well-appointed individual testing rooms, some with one-way mirrors from an observation room to the testing room. Their experience in testing both children and adults, and their research and physical resources are critical for us in running the Adult Study; the Study Day will take place in their Biobehavioral Testing Facility; and 2) Drs. Claire Coles (Emory University) and Therese Grant (University of Washington), who will each recruit a cohort of Adults with FASD and appropriate controls at their sites. We are collaborators on Dr. Coles U01 and she is a collaborator on our U01; all protocols and procedures will be consistent at the three sites.

The past 7 months have been spent writing two IRB (called CREB [Clinical Research Ethics Board] at UBC) applications, meeting regularly with the Oberlander team and having regular monthly phone calls with Drs. Claire Coles and Therese Grant to plan the Adult Study (Aim 2), develop all of the materials needed for the proposed research, hiring and training Research Assistants, ordering the instruments to be utilized with participants on our study day, meeting regularly in person or by phone with colleagues involved in both Aim 1 and Aim 2 to plan for initiation of the studies, and other activities critical for getting our studies up and running. Specifically, our activities include the following:

- All investigators and research personnel completed online UBC and NIH ethics training.
- Working with the Oberlander team, we have written two ethics applications. One application is for collection and processing of blood samples to be obtained from the Vancouver Adult Study and all of the distant sites from which we will receive samples from pregnant women, infants, children and adults with FASD (Ukraine, San Diego, Minnesota, Atlanta, Seattle); the second application is for all of the procedures involved in recruiting and testing adults with FASD in Vancouver. Both have now been approved (H17-01178 and H17-0090).
- We met monthly with the Oberlander team to plan the Adult Study.
- We had monthly phone calls with Drs. Coles and Grant to plan the Adult Study so that all procedures and activities are consistent across our three sites.
- We have had several calls with Dr. Tina Chambers to discuss her Ukraine study and collection of blood samples, as well as the San Diego cohort that we plan to recruit and test in collaboration with Dr. Ken Jones.

Together with Drs. Coles and Grant we developed a Health Survey for the Adult Study. This Survey utilized modules from the BRFSS developed by the CDCP and we wrote original modules to cover areas not covered by the BRFSS but that are critical in assessing adults with FASD; these original modules were based on health surveys developed by various medical groups (e.g., the Arthritis Society). The Health Survey has been finalized and entered into Redcap.

- We obtained a Redcap license for the Vancouver site and are learning to use it.
- We developed protocols and forms for obtaining consent to participate in the Adult Study. These include an interactive version of the consent form to ensure that all individuals understand fully what is being asked and what the study involves. Consent will also include permission to obtain health records and other linked records.
- We worked with Drs. Coles and Grant to finalize the list of instruments/ questionnaires to be utilized on the Study Day; all of these have now been ordered. They include: i) detailed demographic information (Demographic Questionnaire) and descriptive data (exposure category [FAS, pFAS, PAE, Unexposed Contrast Group], date of birth, sex, ethnicity, race; ii) information on physical and mental health (Health Questionnaire that we developed, and that also includes Healthy Days [Health-Related Quality of Life], Health Access, Disability, COPD, Cognitive Impairment, Cognitive

Decline, and Adverse Childhood Experience (ACE)] and the Psychiatric Diagnostic Screening Questionnaire (PDSQ); iii) cognitive/executive function (elements from the NIH Tool Box: Dimension Change Card Sort Test of Executive Function-Cognitive Flexibility, Flanker Test of Executive Function-Inhibitory Control and Attention, Picture Sequence Memory Test of Episodic Memory, List Sorting Working Memory Test and Pattern Comparison Processing Speed Test; iv) activities of daily living/adaptive function (adult version of the Adaptive Behavior Assessment System, Third Edition (ABAS-3); v) environmental factors/activities and participation (Life Stressors and Social Resources Inventory-Adult [LISRES-A]; and vi) Substance Abuse (Alcohol Use Disorders Identification Test [AUDIT] and the Drug Grid (self-report measure of frequency and amount of use for tobacco, alcohol, and a number of drugs).

- We have arranged for access to the Advia 120 Hemaology Analyzer in the Center for Blood Research on the fourth floor of my building, which will be utilized for our hematology screen on a small aliquot of blood from the larger sample to be taken at the end of the Study Day for analysis of plasma cytokine levels.
- We have finalized the list of cytokines to be analyzed on our participants:
    - For the child samples we will utilized Meso Scale Discovery (MSD) human cytokine 40-plex V-PLEX kit (CRP, Eotaxin, Eotaxin-3, FGF (basic), Flt-1/VEGFR-1, GM-CSF, ICAM-1, IFN-γ, IL-10, IL-12/IL-23p40, IL-12p70, IL-13, IL-15, IL-16, IL-17A, IL-1α, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-8 (HA), IP-10, MCP-1, MCP-4, MDC, MIP-1α, MIP-1β, PlGF, SAA, TARC, Tie-2, TNF-α, TNF-β, VCAM-1, VEGF-A, VEGF-C, VEGF-D).
    - For the adult samples, in addition to those listed above, we will measure levels of Th17 cytokines (MSD U-PLEX kit; IL-17A/F, IL-17A, IL-17E/IL-25, IL-21, IL-22, IL-27, IL-29, IL-31, IL-33, TSLP) and early RA biomarkers (EGF, MMP-1, MMP-3, Leptin, YKL-40, Resistin, TNF-R1).
- We have developed posters for recruitment of adult participants and in consultation with Jan Lutke and Brenda Knight (a clinical psychologist who will assist us as needed in aspects of our study) have made a list of venues where these will be posted (community centers and other venues where adults with FASD gather). Note: Our IRB mandated that we could not approach potential participants for this study, but rather, this was to be a community study. We could advertise, and potential participants could then contact us. Once they contacted us we could then connect with them, explain the study, consent them, and proceed as usual.
- I presented an overview of the proposed Adult Study as part of a keynote presentation at the FASD Collaborative Roundtable, Douglas College, New Westminster, BC, Canada, November 25, 2017. Several of the attendees who work in organizations or residential facilities approached me after the talk to say they know individuals with FASD who may want to participate in the study. We exchanged business cards.
- Drs. Coles, Grant and I will present an overview of the Adult Study at the upcoming " 7th International Conference on Fetal Alcohol Spectrum Disorder, Research: Results and Relevance. Integrating Research, Policy and Promising Practice Around the World." March 1-4, 2017, Vancouver, BC, Canada
- Jan Lutke and Brenda Knight are currently working on materials to be used to support and debrief investigators, Research Assistants, and study participants. This is particularly critical in working with individuals with FASD.
- We have had several email interactions with Dr. Peter Hammond with respect to our taking of 2D pictures to be utilized by him as part of the development of standards and algorithms for identifying individuals prenatally exposed to alcohol. He has sent us a protocol and we are working with him and with Drs. Coles and Grant to finalize the protocol.
- We have developed a draft of the logistics for the Study Day (all activities, order of tests, rest periods and food needed, transportation and parking, etc). This will be finalized in the next month.

2) Specific Objectives. Our Specific Objectives for the first half of Year 1 were to get our ethics applications approved, have regular planning meeting/phone calls with the Oberlander team and with Drs. Coles and Grant, develop all of the materials needed for the Adult Study, hire and train Research Assistants, order the instruments/questionnaires to be utilized with participants on our study day, meeting/talk regularly with colleagues involved in both Aims 1 and 2 to plan for initiation of the studies, and carry out other activities critical for getting our studies up and running.

For the second half of Year 1, from January to May, our Objectives are:

For the Vancouver Adult Study (Aim 2):

- As per our IRB mandate, we will arrange a meeting with First Nations/Aboriginal Elders to explain the study, our goals for the Adult Study (Aim 2), and how the research will ultimately provide benefits to the community. Dr. Chris Loock will help us arrange and present our proposed research.
- To finalize the details and logistics of the Study Day.
- To do multiple practice runs of the Study Day, initially with all of us and our Research Assistants, then with adult participants who were not exposed to alcohol *in utero*, and then with a few adult participants with FASD. We have strong relationships with a number of adults with FASD, some of whom already know about our study and wish to participate. We will ask them if they wish to help us test out our procedures.
- With each practice run we will refine our procedures and logistics so that the Study Day will run smoothly.
- We plan to begin recruiting subjects at the end of March/start of April and begin testing after the UBC FASD conference, April 18-21. We hope to recruit and test at least 10 individuals by the end of Year 1.
- To work with Dr. Ken Jones to develop criteria for the examination of adults with FASD. While he is in Vancouver for UBC FASD conference, Dr. Jones will observe and train as needed Drs. Oberlander and Loock so that they can carry out the physical examination of the adult participants in our study.
- To carry out a pilot study to work out the methods for assaying cytokine levels in blood spots.

For the studies in Aim 1:

- We will begin to receive samples from each of the sites where samples are being collected.
- Dr. Jeff Wozniak has made significant progress in his study, and we hope to have the full complement of 60 samples by the end of Year 1 or early in Year 2.

3) Significant Results. We have not yet begun recruitment and data collection for this study.

4) Key Outcomes and Other Achievements. Please see 1) Major Activities for a summary of key outcomes and accomplishments to date.

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?

Training

- 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 a monthly lab 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). Amanda Chao has been a Work-learn student in my laboratory for the past year. She will now transition to working with us as a Work-Learn student on our U01. When she graduates in May, we have offered her a Research Assistant position to support our U01.

- Research Assistants will be funded to take NMED 1117, Basic Venipuncture for Allied Health Professionals at the BC Institute of Technology, Vancouver, BC. They will thus be prepared to take blood samples from participants in the Adult Study.

Professional development - Symposia, retreats & meetings:

- Attendance at weekly Neuroscience Research Colloquia
- Attendance at monthly Neuroscience "Pizza Seminars" where PIs and laboratory members from different laboratories that are part of the Neuroscience Program present their research
- Attendance at weekly Cellular and Physiological Sciences Departmental Seminars
- Attendance at monthly seminar series and journal clubs on various topics including Microbiome and Neuroinflammation
- Attendance and presentations at weekly Behavioral Neuroscience Seminar (Dept. of Psychology)
- Attendance and presentations at local, national and international conferences:
    - Yearly conferences on Fetal Alcohol Spectrum Disorder hosted by UBC Interprofessional Continuing Education, Vancouver, BC
    - Neurobiology of Stress Workshop
    - Annual Research Society on Alcoholism Conferences
    - Annual Brain Development Conferences, Kids Brain Health Network (formerly NeuroDevNet)
    - Department of Cellular and Physiological Sciences (CPS) Annual Research Day
    - Annual Meeting of the International Society for Developmental Psychobiology
    - Annual Meeting of the Society for Neuroscience
    - Annual Meeting of the Society for Leukocyte Biology

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

To date, we have not yet begun data collection for the U01. However, we have presented our work, primarily results of the Developmental Project, CIFASD III, at several events:

We presented posters on findings from our Developmental Project at two meetings. We also presented the child data from our DP at a meeting of the Society for Leukocyte Biology and submitted an abstract on the child data to the RSA 2018 meeting. Please see Poster Abstracts and Presentations below.

I was a keynote speaker at the FASD Collaborative Roundtable at Douglas College, New Westminster, BC, November 25, 2017, where I presented an overview of the Adult Study from our U01.

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:

- Continuing with recruiting and testing of participants in Vancouver for the Adult Study. Our goal is to recruit the full cohort of 120 individuals by the end of year 3 or by early in Year 4.
- To run cytokine assays on a continuing basis as we collect samples. We can run 40 samples on each plate, so can run a plate for each batch of 40 samples collected.
- To receive and assay blood samples from: Ukraine (Dr. Chambers, matched mother-infant pairs); San Diego (Drs. Chambers, Jones, Mattson, matched mother-child pairs and additional blood sample for children born in San Diego County and brought back for examination); and Minnesota (Dr. Wozniak, children with FASD treated with choline or placebo)
- To receive and assay blood samples from adults with FASD and controls from Dr. Coles (Atlanta) and Dr. Grant (Seattle).

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

No publications from the current U01 to date.

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

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. Altered maternal immune networks are associated with adverse child neurodevelopment: Impact of alcohol consumption during pregnancy. *Biological Psychiatry*, Submitted. CIFASD manuscript submission template completed November 2017 and updated January 2018 (Concept Proposal #41).

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.

***Poster Abstracts and Presentations**

Bodnar, TS, Raineki, C, Wertelecki, W, Yevtushok, L, Zymak-Zakutnya, N, Honerkamp-Smith, G, Wells, A, Chambers, CD, Weinberg, J, and the CIFASD. Identifying an Immune Signature Characteristic of Fetal Alcohol Spectrum Disorders. Society for Leukocyte Biology, October 5-7, 2017, Vancouver, BC, Canada.

Bodnar, TS, Raineki, C, Wertelecki, W, Yevtushok, L, Plotka, L, Zymak-Zakutnya, N, Wells, A, Honerkamp-Smith, G, Coles, CD, Kable, JA, Chambers, CD, Weinberg, J, and the CIFASD. Cytokine disturbances associated with prenatal alcohol exposure in children: Implications for health and development. Submitted for presentation at Research Society on Alcoholism, June 16-20, 2018, San Diego, CA.

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

- Chambers will provide us with plasma samples from pregnant women and their infants at birth (alcohol-exposed, alcohol-unexposed) and clinical data from the Ukraine birth cohort, as well as statistical support in analyzing data and support in writing manuscripts.
    - We regularly communicate by email or phone to discuss the research.
- Jones and Chambers will provide us with plasma samples from children (alcohol-exposed), as well as maternal (mid-pregnancy) plasma samples and child newborn blood spots; health questionnaire also completed and data provided. Control (unexposed) children will be recruited in collaboration with Mattson (as per Aim 1 of her U01) and plasma samples and health questionnaire data obtained through Jones and Chambers. Physical examination and blood sampling on all alcohol-exposed and unexposed children by Dr. Jones.
    - We have had several phone calls with Drs. Chambers and Jones to discuss the research and logistics of the study.
- Wozniak will provide us with plasma samples from children being recruited to his new choline study (this collaboration not part of Wozniak's CIFASD U01).
- Coles and Grant are collaborating closely with us. All of our testing procedures are consistent with each other. This will allow us to analyze data from each site independently as well as combine the data from our three cohorts for analysis. Drs. Cole and Grant will provide us with plasma samples from adults (in Atlanta and Seattle cohorts, respectively, as per their U01) with FAS, pFAS/physical effects of alcohol, ARND, unexposed controls, as well as diagnosis, demographic information, information on physical and mental health, substance use, cognitive and adaptive function.
    - We have regular monthly phone call to discuss the study, develop and share materials, plan logistics, etc.
- Weinberg to provide 2D facial pictures of subjects to Hammond for his analysis, which involves bringing 2D photo data into the 3D model.

    - We have had several email exchanges to discuss the protocol and logistics for taking the pictures and sending them to the UK.

- Aliquots of plasma from blood samples taken from infants in the new Ukraine birth cohort (Dr. Chambers' U01) 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.
- Weinberg and Blanchard will interact and share data over the course of their studies. Dr. Blanchard's animal model studies 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 Dr. Blanchard in the interpretation of his microbiome data from maternal females and offspring. Finally, new data to be collected in our proposed U01 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 to alcohol/non-exposed 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, Dr. Blanchard and I will interact extensively once samples have been collected and analyzed.

If a project you were the PI on was part of Phase III of CIFASD, use this space to update the Advisory Committee on any new progress not previously reported.

Our Developmental Project was part of CIFASD III. Results from this project are presented in the various sections above.

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

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).

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).

Aim 3. Fully characterize relationships between connectomics and cognitive 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.

- Although not a Specific Aim for my project, an aim for Dr. Joanne Weinberg's project involves examining immune factors from samples of humans with FASD. We are obtaining blood samples as part of Dr. Wozniak's R01 project and providing them to Dr. Weinberg in support of her project.

- Similarly, not a Specific Aim for my project, but an aim for Peter Hammond's project involves collecting 3D facial images of children with FASD and controls. We are supplying him with images.

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

No, the goals have not changed.

B.2 What was accomplished under these goals?

1) Major Activities.

- IRB approval obtained
- Hiring completed (two new research coordinators and a 50% MRI technician)
- Coordinators trained, including travelling to Dr. Mattson's site at SDSU for formal training on the cognitive battery used in our joint projects
- MRI acquisition protocol finalized and tested; Image analysis pipeline developed
- Acquisition of 3D facial images for Dr. Peter Hammond's project upgraded (new 3D camera system obtained, installed, and training completed)
- Blood samples obtained from Dr. Wozniak's R01 project (R01AA024123-01) to provide to Dr. Weinberg's CIFASD project; Collected thus far = 16
- Participant recruitment initiated (letters mailed, advertisements initiated, calls made)
- Participants enrolled = 6; Participants with completed MRIs, 3D facial images, and neurocognitive testing = 4

2) Specific Objectives.

- Year 1 (7/1/2017 – 5/31/2018) total target = 30 (approximately 7 or 8 per month until 5/31/2018)

3) Significant Results.

Data collection is underway, so significant results are not yet available from this project. However, we are actively analyzing data from the previous phase of CIFASD (CIFASD Phase III) and publishing.

4) Key Outcomes and Other Achievements.

Currently on target for recruitment now that the initial preparation has been completed and participants are being enrolled.

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?

The research is not intended to provide training and professional development opportunities.

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

Wozniak, J.R. (2017). Fetal Alcohol Spectrum Disorders: From brain imaging to neurodevelopmental intervention. Invited talk given at the University of Calgary's Owerko Centre at Alberta Children's Hospital Research Institute, Calgary, Alberta, Canada.

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

- Recruitment and collection of MRI data, neurocognitive data, and 3D facial images will continue.
- Cognitive data will continue to be collected and shared with Dr. Sarah Mattson's projects.
- 3D facial images will be continue to be collected and shared with Dr. Peter Hammond's project.
- Blood from Dr. Wozniak's R01 study on choline intervention will continue to be shared with Dr. Joanne Weinberg as part of her CIFASD4 project.

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

NIH Public Access Compliance	Citation
Non-compliant	Uban KA, Herting MM, Wozniak JR, Sowell ER; CIFASD.. Sex differences in associations between white matter microstructure and gonadal hormones in children and adolescents with prenatal alcohol exposure. Psychoneuroendocrinology. 2017 Sep;83:111-121. doi: 10.1016/j.psyneuen.2017.05.019. Epub 2017 May 26. PubMed PMID: 28609669.
Complete	Wozniak JR, Mueller BA, Mattson SN, Coles CD, Kable JA, Jones KL, Boys CJ, Lim KO, Riley EP, Sowell ER; CIFASD.. Functional connectivity abnormalities and associated cognitive deficits in fetal alcohol Spectrum disorders (FASD). Brain Imaging Behav. 2017 Oct;11(5):1432-1445. doi: 10.1007/s11682-016-9624-4. PubMed PMID: 27734306; PubMed Central PMCID: PMC5389933.
Complete	Suttie M, Wetherill L, Jacobson SW, Jacobson JL, Hoyme HE, Sowell ER, Coles C, Wozniak JR, Riley EP, Jones KL, Foroud T, Hammond P; CIFASD.. Facial Curvature Detects and Explicates Ethnic Differences in Effects of Prenatal Alcohol Exposure. Alcohol Clin Exp Res. 2017 Aug;41(8):1471-1483. doi: 10.1111/acer.13429. Epub 2017 Jul 10. PubMed PMID: 28608920; PubMed Central PMCID: PMC5563255.
Complete	Gross LA, Moore EM, Wozniak JR, Coles CD, Kable JA, Sowell ER, Jones KL, Riley EP, Mattson SN; CIFASD.. Neural correlates of verbal memory in youth with heavy prenatal alcohol exposure. Brain Imaging Behav. 2017 Jun 27. doi: 10.1007/s11682-017-9739-2. [Epub ahead of print] PubMed PMID: 28656347; PubMed Central PMCID: PMC5745322. [Epub ahead of print]
Complete	Hendrickson TJ, Mueller BA, Sowell ER, Mattson SN, Coles CD, Kable JA, Jones KL, Boys CJ, Lim KO, Riley EP, Wozniak JR. Cortical gyrification is abnormal in children with prenatal alcohol exposure. Neuroimage Clin. 2017 May 22;15:391-400. doi: 10.1016/j.nicl.2017.05.015. eCollection 2017. PubMed PMID: 28580296; PubMed Central PMCID: PMC5447653.

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

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. (under revision). Two-year cortical trajectories are abnormal in children and adolescents with prenatal alcohol exposure. *Developmental Cognitive Neuroscience.*

***Poster Abstracts and Presentations**

Wozniak, J.R. (2017). Brain imaging and connectivity: An adjunct to screening. Plenary session presentation at the 7th International Conference on Fetal Alcohol Spectrum Disorders Research: Results and Relevance, Vancouver, BC, Canada.

Inkelis, S.M., Thomas, J.D., Coles, C.D., Kable, J.A., Wozniak, J.R., Riley, E.P., Mattson, S.M., and the CIFASD. (2017). The relationship between sleep problems and behavioral functioning in 5-year olds with prenatal alcohol exposure. Poster presented at the annual meeting of the Research Society on Alcoholism, Denver, CO.

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 will assist Dr. Mattson in testing the Decision Tree software in our FASD Clinic.
- We continue to collect 3D facial images to be shared with Dr. Peter Hammond's project. We are working on a collaborative publication with Dr. Hammond and Mike Suttie at present.
- 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 CIFASD4project.
- Dr. Christie Petrenko will be collecting focus group data for her CIFASD4 project in the Spring, 2018. We have initiated a connection with our local state organization (MOFAS) to facilitate recruitment for Dr. Petrenko's project and we will be distributing materials to our research participants as well.

If a project you were the PI on was part of Phase III of CIFASD, use this space to update the Advisory Committee on any new progress not previously reported.

Analysis of CIFASD Phase III data continues and publications are under revision as well as in preparation.

Principal Investigators: Thomas Blanchard and Sandra Mooney
Institution: University of Maryland School of Medicine
CIFASD4 Project Title: Prenatal alcohol effects on the gut microbiome contributing to failure to thrive and altered immune function
Grant Number: UH2AA026109

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? No.

B.2 What was accomplished under these goals?

1) Major Activities. 2) Specific Objectives. 3) Significant Results.

4) Key Outcomes and Other Achievements. Preliminary studies used qRT-PCR to evaluate a gross measure of fecal microbiota composition in control and ethanol-exposed animals. Specific taxa included in that analysis were Bacteroidetes, Firmicutes, Gammaproteobacteria, as well as a universal primer pair that targets all microbiota in the taxa being studied including Bacteroidetes, Firmicutes, Actinobacteria and the α and γ subdivision of Proteobacteria By comparing the relative amounts of each targeted taxa to that amplified by the universal primers, a shift in ratio of specific taxa can more easily be identified than when determining what percentage a taxa represents of the total bacterial populations. For these preliminary analyses however fold change of each taxa was determined and assessed using 2^(-delta deltaCT).

Stool samples were collected from 7 dams (4 control (Con), 3 ethanol (Eth)) for this preliminary analysis of microbiome changes. Samples were collected on gestational day (G)6 prior to beginning the liquid diet and again 14-16 days later just before dams were returned to chow. Because this was preliminary data, samples were collected from animals used in ongoing experiments and the ethanol content of the diet was 3% between G6 and G21. Control animals received liquid diet containing maltose as a caloric replacement for ethanol. All animals had ad libitum access to the liquid diet and water throughout the course of the experiment.

We used qRT-PCR of 16S rDNA to determine if there were diet-induced changes in each bacterial taxa. The taxa chosen were previously determined to be representative of major phyla and groups in laboratory rodents. Data were generated as the mean number of cycles to threshold (CT) for each group at both time points. DeltaCT (the difference in CT between G6 and G21) shows the change in the amount of a particular taxon across time but within a nutritional group. Delta deltaCT was the difference in deltaCT for the two nutritional groups, and was used to calculate fold change (2^(-delta deltaCT)).

In Con animals, there was an increased deltaCT for Bacteroidetes, Firmicutes, and Gammaproteobacteria showing a reduction in the amount of these between G6 and G21., The universal primers however, representing the sum total of Bacteroidetes, Firmicutes, Actinobacteria and the α and γ subdivision of Proteobacteria revealed an increase during the same time.

In Eth animals, deltaCT decreased for Bacteroidetes, Firmicutes, and the universal primers, showing increases in each, but Gammaproteobacteria showed a decrease similar to that seen in control animals.

Fold change analysis allows comparison of the change in the ethanol-exposed animals with the change in control animals across time. The change in the universal primers was almost 5 fold higher in Eth animals. Bacteroidetes was ~16-fold higher, Firmicutes ~3.5-fold higher, and Gammaproteobacteria was only 0.59-fold higher (i.e., essentially the same).

We also ran the same preliminary analysis using samples from pups from Con or Eth dams. Dams were returned to chow on G21 prior to birth and maintained on it through weaning (postnatal day(P)21). Pups were housed in same-sex groups with littermates and samples were collected on P30.

The deltaCT for each taxon was between -1 and 1, suggesting that persistent changes may be relatively minor. However, it is likely that these data are confounded by having all nursing and post-weaning animals on lab chow.

We propose modifying our experimental protocol to have female rats fed a liquid diet for ~8 weeks prior to mating and to keep all animals on their assigned liquid diet throughout the course of the experiment.

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?

IACUC has approved changes to the animal protocol and rats have been ordered. Rats will be fed a liquid diet as in the modified model described in B2. Stool samples will be collected and DNA will be extracted and stored until use. GI tissue will be collected and stored until use. Spleen will be analyzed as described in Aim 3. All analyses will be completed in FY02.

***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.

This project is expected to interact with the informatics core (U24, Barnett) after the microbiota data are collected. It may also inform two of the U01 projects: microbiome data (Chambers) and immune data (Weinberg). There is nothing to report at this time as experimental data have not been collected yet.

Principal Investigators: Kazue Hashimoto-Torii and Masaaki Torii
Institution: Children's National Medical Center
CIFASD4 Project Title: Biomarker for Intellectual Disability in Children Prenatally Exposed to Alcohol
Grant Number: UH2AA026106

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

Aim 1: Establish novel peripheral biomarkers for predicting the risk of cognitive and learning deficits in **mice** prenatally exposed to ethanol.

Aim 2: 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?

We compared several analytic pipelines for both Aims 1 and 2, and determined the best ones. With these pipelines, we obtained ~30,000 genes including expected genes in each cell type (T cell, B cell and monocytes). ~30 RNA sequencing experiments by single ended Highseq with 5 million reads were performed with the FASD model and control mice. These animals were also placed for behavior tests. Different experimenters conducted behavior tests, RNA collection, and library preps to ensure the blindness for experimental groups and unbiased analyses. Currently, another experimenter is processing read demultiplexing/alignment/counting. Another set of animals (~10) are in the process of behavior tests and RNA collections. We are also coordinating the procedures to obtain blood samples of human patients from Dr. Chambers' group.

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?

We plan to complete all the wet analyses (mouse RNA sequencing and behavior tests) for the Aim 1 by the end of March, 2018. We will continue bioinformatics analyses to define biomarkers.

We anticipate to obtain the first human blood samples from Dr. Chambers' group by the end of the first year to perform analyses for Aim 2.

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

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

Shahid Mohammad, Li Wang, Seiji Ishii, Peijun Li, Toru Sasaki, Zenaide Quezado, Fumiaki Imamura, Hirotaka Nishi, Joshua Corbin, Judy Liu, Yuka Imamura Kawasawa, Masaaki Torii, Kazue Hashimoto-Torii. Reduction of excessive KCNN2 ameliorates the learning disability in the mouse model of Fetal Alcohol Spectrum Disorders. In preparation.

*** 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 aims at identifying biomarkers for intellectual disability in children prenatally exposed to alcohol, which is directly related to the aims of CIFASD to definitively outline a diagnostic schema toward the development of effective intervention and treatment for FASD.

The Administrative Core helps to facilitate communications and collaborations with other research programs. The Dysmorphology Core will perform physical exams and validate the data for the patients in the Ukraine study. Their clinical data can be associated with our biological data. The Informatics Core will provide access to a large amount of clinical data collected from the Ukrainian cohort. The Informatics Core will also make the Central Repository available for our collected data and provide access to the computational resources at IUPUI for analysis as needed. We will collaborate with NOFAS Outreach Core to disseminate key research findings to non-research community. Our project is closely tied to the U01 projects of Drs. Chambers and Weinberg. The miRNA biomarkers (Chambers) and cytokine biomarkers (Weinberg) will be tested in parallel to our biomarker study using the blood samples from the same patients in the Ukraine cohort of Dr. Chambers. The serums separated from the white blood cells in our study will be preserved for the future use, such as for the potential collaborations with the group of Dr. Foroud for genetics studies. The biomarkers examined in our study will also be examined in the complementary fish and mouse models of early-gestational alcohol exposure (proposed by Drs. Eberhart and Parnell) to extend our findings with the mid-gestation-equivalent model.

We have had Skype meetings regularly with Dr. Chambers' group. The topics included 1) how to overcome difficulties in transferring human blood samples to be used for RNA collections, 2) progress in the process of IRB approvals, and 3) the process of obtaining the cohort information. The IRB protocol was successfully transferred from Dr. Chambers' group to the groups of Drs. Hashimoto-Torii and Torii.