CIFASD4
Late Fall 2020
Progress Reports

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Principal Investigator: Edward Riley
Institution: San Diego State University
CIFASD4 Project: Administrative Core of the CIFASD
Grant Number: 5U24AA014811-17

Specific Aims

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.

Accomplishments

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

2) Specific Objectives. The AdminC Specific Aims are stated above.

3) Significant Results. The AdminC is not responsible for conducting any research studies, but rather facilitates the integration and efforts of the other CIFASD components.

4) Key Outcomes and Other Achievements.

The Spring 2020 CIFASD Progress Meeting, organized and facilitated by the AdminC, was held via Zoom on June 23rd and 25th. Presentations focused on progress since the last face-to-face meeting in October 2019, exciting new data, and deliverables. PIs also shared any impacts of COVID-19 might have had on their research. There was a group discussion, minus NIAAA staff, on thoughts about a potential CIFASD5. Led by Drs. Riley and Charness the focus was on what the next iteration of CIFASD should address, what themes and ideas should continue, what resources the consortium should try to recruit or expand, what the important issues and questions the field faces, and how CIFASD might direct its objectives and expertise to those issues. Preparations are underway for the Late Fall 2020 CIFASD Progress Meeting which will be held during select hours on December 2nd, 3rd and 4th via Zoom.

While referencing the June progress presentation slides, April NIH progress reports (RPPRs), and information from the Google Drive progress tracking tables, the Science Advisory Board (SAB) completed reviews of the progress of the various projects over the summer. They were then summarized and edited

by Dr. Hannigan, and comments on the consortium as a whole and project specific feedback were emailed to each PI by Dr. Riley in August. These reports were also reviewed by Drs. Wang and Dunty at NIAAA.

Dr. Riley assisted Drs. Wang and Dunty by collecting and collating detailed follow-up information from all PIs on the impact of COVID-19 on the consortium projects. Drs. Riley and Charness also responded to a request from Dr. Wang to summarize the most significant accomplishments by CIFASD since its inception and since the start of CIFASD4, as well as details on spin-off projects and young investigators.

The AdminC continues to coordinate, convene, and moderate the monthly meetings of CIFASD4. Agendas are prepared by the AdminC along with input from CIFASD PIs, the SAB, and NIAAA staff. The platform for these meetings transitioned in the spring from WebEx to Zoom. Notably in recent months, Dr. Riley has been coordinating and recruiting speakers for themed presentations at the top of the agenda. In July, the theme was stigma and FASD and Sylvia Roozen, an FASD researcher from Maastricht University, was invited to give a talk on her recent publication in *Foundations of Science* on this topic. Follow-up talks on stigma were given by Dr. Jones, Tom Donaldson of NOFAS, and Dr. Dunty. In August, Olivia Weeks, a postdoctoral fellow at Harvard Medical School and Brigham and Women's Hospital, was invited to present on her research involving the metabolic health outcomes following prenatal alcohol exposure (PAE) in humans and zebrafish. A companion talk followed on cardiovascular health and PAE and alterations in insulin by CIFASD investigator Julie Kable. In October, Dr. Coles provided a preliminary report on alcohol-affected adults' self-reported health and mental health status. Dr. Mattson also provided a walk-through of the BRAIN online neurodevelopmental testing tool that has now been deployed to subjects. In November, an invited guest, Jessica Bomyea of UCSD, presented on executive functioning training to address cognitive and emotional dysfunction. Each monthly meeting also includes time for pertinent announcements, updates from the committees, and comments from the SAB and NIAAA.

During the past few months, the Data Access Committee (DAC) has received external data use access requests through the revamped online Data Sharing 2.0 system. Requests are distributed to the committee members and reviewed in a timely manner. Committee meetings are arranged with assistance from the AdminC to allow the DAC the dedicated time to make their make final determinations. Dr. Thomas serves on the DAC committee and the chair, Dr. Wetherill, consults with Dr. Riley as needed and prior to the final decisions being communicated to the requester. Dr. Mattson, chair of the Publications Policy Committee, provides summaries during each monthly meeting highlighting new concept proposals, manuscript submissions and papers that have been accepted for publication. These summaries are prepared with assistance from the AdminC responsible for maintaining all of the online Google forms used to collect this information from the consortium. Dr. Thomas also serves on this committee and Dr. Riley provides advisement on any publication issues that arise.

The AdminC continues to maintain the CIFASD.org website, which highlights new consortium research findings and the Google Drive progress tracking tables, which are utilized to evaluate project progress and assess whether additional support or resources may be needed for the project to deliver on their aims. The AdminC also monitors and controls the allocation of shared consortium resources, such as the portable Canfield Vectra 3D handheld Canon cameras, and a system was recently delivered to Drs. Jones and Chambers. The system used in the Ukraine has been not functioning properly and the handheld system will be beneficial to that research site once travel to that area resumes.

The typical promotion of CIFASD findings by Dr. Riley at various national conference and international meetings has been halted since March by the ongoing pandemic. The 9th International Research Conference on Adolescents and Adults with FASD scheduled to be held in Vancouver, Canada in April was canceled, and the Alcoholism and Stress meeting scheduled for May in Volterra, Italy was postponed to 2021. The in-person joint RSA Scientific Meeting/ISBRA Congress scheduled to take place in New Orleans, LA in June was also canceled; select portions of the program, such as poster presentations, were moved to an optional online platform. The CIFASD-themed symposium entitled, "CIFASD studies on the role of genetics in FASD," organized by Dr. Charness that was accepted for 2020 meeting will be moved forward to the 2021 meeting in San Antonio, TX. The speakers will include Drs. Amanda Mahnke (Miranda lab), Eberhart, Parnell, and Foroud. Similarly, the CIFASD-themed symposium entitled, "Using technology to move forward on the recognition and treatment of FASD," submitted for presentation consideration at the European FASD Alliance (EUFASD) research conference scheduled to take place in Arendal, Norway September was

postponed to 2021. Organized by Drs. Riley and Charness, the speakers include Drs. Riley, Suttie, Mattson and Petrenko.

CIFASD research was featured in the Fall 2020 issue of *NIAAA Spectrum*, an online magazine. The feature articled highlighted recent findings by CIFASD investigators including Dr. Hashimoto-Torii's work on molecular mechanisms and motor deficits, Dr. Wozniak's studies on choline supplementation, and Drs. Petrenko and Tapparello's intervention app. In the near future, Dr. Riley will be the leading expert for a *Psychwire* ASK online Q&A session about fetal alcohol syndrome, and Dr. Charness will be delivering a talk containing highlights of CIFASD research at the NIAAA 50th Anniversary Science Symposium webinar.

Nine new publications citing CIFASD funding have been published since the June progress meeting, bringing the total number of publications citing CIFASD grants as a source of support in 2020 to 24, and the overall total number of publications since the consortium's inception to 299. With regard to the AdminC, the grant has been cited as a source of support in 107 papers, 32 since the start of CIFASD4.

Through the AdminC subaward with Blue Resonance, LLC, Dr. Chockalingam continues to assist the consortium with its CIFASD4 aim of developing and utilizing mobile apps and online eHealth technologies. He has been busy assisting Dr. Mattson with the BRAIN (formally FONS) neurobehavioral data collection app. He also continues to maintain updates and support as needed for her decision tree tablet app and web portal interface. He meets regularly with Dr. Riley to discuss the development of automated diagnostic tools deployable as mobile apps to assist in the diagnosis of FASD. They submitted a Phase II STTR application for an idea that was a spin-off of these communications which will be reviewed this fall.

The FFR for the previous budget year was recently accepted by NIAAA and a carryover request will be submitted before the end of year by the AdminC.

Additional Questions

CIFASD4 Synergy. *Describe your project's interrelation with aims of the CIFASD consortium its other projects and the progress on those interactions.*

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

Pandemic - May 2021. *If nothing changes with regard to the pandemic, what do you hope your project can achieve by May 2021 (the end of the 4th year budget period for CIFASD4) and/or what Covid-19 impacts will remain obstacles*? n/a

Administrative Supplements. *For this reporting period (June 2020 to present), please list any Administrative Supplement associated with this award and a sentence or two about what it entails.* n/a

Publications June 2017 - present

Publications citing the AdminC U24AA014811 as a source of support [Accepted & In Press]

Key: **Bold = AdminC Author** Underline = Pre-CIFASD4 Developmental Project/AdminC Subaward PI

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Principal Investigator: Kenneth Lyons Jones
Institution: University of California, San Diego
CIFASD4 Project: Dysmorphology Research Resource
Grant Number: 5U24AA014815-17

Specific Aims

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.

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.

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.

Accomplishments

1) Major Activities:

Aim #1: Because of the pandemic, no subjects were seen at any clinical site in CIFASD.

Aim #2: Our efforts to train Pediatric residents using telemedicine has not been possible because of the pandemic. We are hopeful that this will be possible in the second half of Year 4 and Year 5 of CIFASD4. However, we are actively involved in establishing a system to evaluate subjects at outlying clinical sites in Alaska.

Aim #3: We continue to see patients at our UCSD/Rady Children's Hospital FASD Clinic (1/2 by telemedicine and 1/2 Face-To-Face) albeit at a decreased volume.

2) Specific Objectives:

- To diagnose FASD at all CIFASD project sites where new subjects are being recruited.
- To train physicians and other health care providers throughout the world with expertise in evaluating an individual for FASD.
- To increase the number of research subjects available to support the CIFSD Consortium's research projects and overall mission through subjects ascertained at out UCSD/Rady Children's Hospital Research Registry.
- To establish Telemedicine as a valid and reliable method to diagnose FAS/D.

3) Significant Results:

Aim #1: No subjects were evaluated at any of the Clinical sites in CIFASD.

Aim #2: Completed a paper entitled "The Use of Telemedicine for the Physical Examination of Fetal Alcohol Spectrum Disorder" and submitted it on September 16, 2020 to *Alcoholism: Clinical and Experimental Research*. It is now in final review. The study documented that telemedicine is a valid and reliable method for examination of the physical features of FASD.

Aim #3:

- We have recruited 20 subjects prenatally exposed to alcohol seen in our UCSD/Rady Children's Hospital FASD Clinic for our Research Registry for a total of 250 subjects recruited overall.
- We have recruited and referred 27 subjects to Dr. Sarah Mattson's U01 Decision Tree at SDSU.
- No additional specimens have been sent to Dr. Joanne Weinberg's U01.
- No additional families have been sent to Dr. Christie Petrenko's U01.

4) Key Outcomes and Other Achievements:

The development of methods to achieve more precise measurements and more objective assessments of the physical features of FASD would contribute to wider access to earlier and more reliable diagnoses of the physical component of FASD. It is important to remember that minor differences in the evaluation of

these features can lead to a divergence in the final assessment of whether the facial component of the diagnosis of FAS or pFAS is or is not present, and ultimately whether the child does or does not have FAS or pFAS. Our colleagues in CIFASD, Dr. Ed Riley and Dr. Ganz Chockalingam, have developed the app Morpheus Q, aimed at providing a far more accurate and objective evaluation of the cardinal facial features of FASD. The measurement of the palpebral fissure length (PFL) currently relies on a manual method that requires placing a hard ruler at the optimal distance from the eyes of the subject, choosing the correct angle so the ruler is parallel to the face at the level of the eyes, and selecting the exact points at the inner and outer canthi as boundaries. Given these measurements often differ by 1 mm or more among different examiners and even for the same examiner in two different exams, we propose that taking a 3D photograph with a handheld device using the Morpheus Q app will improve precision and reduce variability when measuring the PFL. In addition, the quantification of qualitative physical features such as the smoothness of the philtrum and the thinness of the vermilion border of the upper lip is not at this time using continuous scales, but rather comparison with a 5-point Likert scale with a single image for each score 1-5. The assessment is in many cases subjective, and would benefit from a continuous scale for both features also included in the Morpheus Q app. These improvements in the objectivity and accuracy of the identification of the facial features are particularly important in underserved areas where expertise in diagnosis of this disorder is lacking.

In Year 5 of CIFASD4, we propose to develop a collaboration with officials in Alaska to evaluate these new tools to assist the diagnostic evaluation of individuals for FASD. The state of Alaska has a network of diagnostic teams for FASD, which are funded by the Division of Behavioral Health and overseen by the Office of Substance Misuse & Addiction Prevention (OSMAP- Division of Public Health). In addition, the geographic and demographic characteristics of Alaska indicate that the development of tools for telehealth and remote assistance in diagnosis, and potentially also in treatment, could have a high impact in access to optimal healthcare for FASD.

Toward this end, we have had a number of conversations with Ryan Ray, Marilyn Pierce-Bulger, Interim President, Alaska Center for FASD and Hope Finkelstein, FASD Program Manager, Office of Substance Misuse & Addiction Prevention, Department of Health and Social Services, State of Alaska. In addition, Dr. del Campo spent one week in Alaska in early September interacting with these individuals and visiting in various doctors' offices. A plan for the next year is materializing. Hope Finkelstein has agreed to invite several of the FASD providers in the state to try the app. Dr. Ganz Chockalingam has communicated with her and has agreed to create a custom version of the app for Alaska and, when done, he will share a link to download it with the providers in Alaska. Once they have installed the app on their phone, a zoom call will be set up and Dr. del Campo and Dr. Chockalingam will walk them through the features. Dr. Jones and Dr. del Campo will schedule a real time telemedicine training to ensure expertise in both standard assessments of the facial features with a ruler and the lip-philtrum guide, as well as with the use of the different features of the Morpheus Q app.

This also brings up the possibility of using the 3D image the app takes as a mode of teleconsultation. This refers to the possibility of evaluating together with the providers a 3D image taken by the app. On our end, we could conduct the evaluation looking at the photo with 3D Oculus goggles real time or later. These plans, which will require specific IRB authorizations to share images, are currently being discussed with our Alaskan partners.

Additional Questions

CIFASD4 Synergy. *Describe your project's interrelation with aims of the CIFASD consortium its other projects and the progress on those interactions.*

- In that all children in all CIFASD clinical sites are evaluated using a standard protocol by one of two dysmorphologists in the Dysmorphology Research Resource (DDR) with expertise in recognition of features characteristic of prenatal alcohol exposure increases the integrity of the conclusions that can be drawn regarding the overall aims of the CIFASD Consortium.
- The fact that those two dysmorphologists are extremely involved in training physicians and other healthcare providers to perform an accurate diagnosis of FASD increases the pool of physicians who are capable of diagnosing the disorder.

- The DRR, through our UCSD/Rady Children's Hospital FASD Clinic, has recruited over the last 3 and one-half years 250 subjects, all of whom were prenatally exposed to alcohol. Many of those subjects have been used by CIFASD investigators for their research projects. In addition, a number of them have received blood and/or urine specimens taken from other subjects we have recruited that have been stored in our Research Biorepository and have subsequently been sent to them.
- The development of a valid and reliable telemedicine program that can be utilized in underserved areas of the U.S., far from physicians with expertise in diagnosis of FASD provides potentially early-identification of this disorder to countless individuals for whom that diagnosis would never have been made. In addition to that being important to the individual, the opportunity for investigators to have subjects evaluated by one of two dysmorphologists with expertise in FASD adds greatly to the integrity of their study

Pandemic - May 2021. *If nothing changes with regard to the pandemic, what do you hope your project can achieve by May 2021 (the end of the 4th year budget period for CIFASD4) and/or what COVID-19 impacts will remain obstacles?*

- We think it unlikely that we will be able to evaluate subjects at any of the clinical sites in the U.S., Canada or Ukraine either by Telemedicine of Face-to-Face. That however will require discussions with the PIs at the clinical sites.
- If things continue in Alaska in the way they are moving now, it is quite likely that we will be able to schedule in real time telemedicine training with physicians and other healthcare providers in Alaska to ensure expertise in both standard assessments of the facial features with a ruler and lip-philtrum guide, as well as with the different features of the Morpheus Q app. We will then be able to develop a cadre of physicians and other healthcare providers in Alaska that will allow us to test the Morpheus Q App as well as the ability of the physician and other healthcare providers to use the 3D image the app takes as a method of telecommunication.
- We will continue to see patients by Telemedicine and Face-to-Face at our UCSD/Rady Children's Hospital FASD Clinic and will continue to add subjects to our Research Registry. In addition, we will continue to recruit and refer subjects to Dr. Sarah Mattson's U01 Decision Tree at SDSU and to other clinical studies when they are requested.

Administrative Supplements. None

Publications June 2017 - present

Publications [Accepted & In Press]

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Principal Investigator: Christina Chambers
Institution: University of California San Diego
CIFASD4 Project: Early Predictors of FASD in Ukraine
Grant Number: 5U01AA014835-17

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

Accomplishments

1) Major Activities.

Three biomarkers in 200 newly recruited mothers and infants are proposed to be assessed. Two of these biomarkers require blood samples (miRNA and markers of inflammation). The third biomarker is the cardiac orienting response. As of November 1, 2020, 140 pregnant women of the target sample size of 200 pregnant women have been recruited and maternal blood samples in the mid-trimester and third trimester have been obtained. Among these pregnant women, 136 unique pregnant women have completed one or more 2D ultrasound scans. Of the infants who have already been born, we have captured blood samples from 4, and we are still awaiting subsequent visits to obtain blood samples from the remainder who will consent. Among school-age children in the cohort, 55 blood samples have been collected from 7-10 year-olds (dried blood spots some venous blood samples). Neurobehavioral testing has been completed for 30 six-month old infants, 11 twelve-month old infants in the new cohort. To date, 96 school-age children have completed neurobehavioral testing or about 2/3 of the target sample size of 150. 3D images from the site where the camera resides have been captured for 35 children. This represents since our last report which covered data collected through February, 2020, 13 new mothers recruited, 2D ultrasounds from 9 new mothers, blood sampling from one additional infant and one additional child tested at 12 months of age. Recruitment has been suspended due to COVID-19 during the March/April-October, 2020 period.

With respect to the third biomarker, the cardiac orienting response paradigm (COR), analysis of data previously collected for this measure has been ongoing. Under funding from a separate R01, the platform for wireless transmission and scoring has been developed and has been tested in Ukraine. An adaptation of the wireless data capture tool was required, and this has been completed and the product shipped to Ukraine for implementation. This, along with a functional near infrared spectroscopy (fNIRS) measure also being deployed in the Ukraine sample under separate R01 funding, will add to the components of potential value in accomplishing this Aim.

2) Specific Objectives.

Our objectives during this period have been to continue to accumulate data and samples that can be used to address Aim 1 which are required to address Aim 2. For Aim 3, we have provided samples and data to Drs. Suttie (3D images), Noble (2D ultrasound), Torii (child blood samples), Weinberg (maternal and child blood samples).

3) Significant Results.

During this time period enrollment and data collection have come to a halt as research activities have been suspended due to the high prevalence of infection with COVID-19 in the two study sites in Ukraine

In this interim period, we have concentrated on preparing publications that use existing data and samples. These have included one paper demonstrating that there are fetal sex differences in microRNA profiles in mothers following in utero exposure to alcohol (Salem et al, 2020).

We have prepared or submitted two papers on the Cardiac Orienting Response (COR). One demonstrated that the COR captured in infancy can predict later FASD classification based on evaluation at preschool age. An index of deviation from the typical heart rate deceleration during a cardiac orienting process was derived using the entire sample of COR responses collected in Ukraine at 6 and 12 months of age (n=931). The predictive validity estimate resulted in an area under the curve value of 0.765 for predicting to pFAS/FAS status (Kable et al, submitted). A second paper demonstrated in both a U.S. sample and the Ukraine sample that the COR is adequately sensitive and specific to identify children who will go on to have neurodevelopmental deficits (Coleman et al, in preparation).

An additional paper led by a trainee under mentorship of PI Dr. Petrenko examined parental partner influence as a factor in maternal alcohol consumption and depressive symptoms, with subsequent effects on infant neurodevelopmental outcomes (Kautz et al, submitted).

4) Key Outcomes and Other Achievements.

We have strategized with Ukrainian partners about how and when recruitment and evaluations can resume. One of the sites (Rivne) which does not provide prenatal care is working with a local obstetric provider to encourage referrals to the Rivne Diagnostic Center where women can be screened and enrolled.

Additional Questions

CIFASD4 Synergy. *Describe your project's interrelation with aims of the CIFASD consortium its other projects and the progress on those interactions.*

We have provided samples and data to Drs. Suttie (3D images), Noble (2D ultrasound), Torii (child blood samples), Weinberg (maternal and child blood samples).

Pandemic - May 2021. *If nothing changes with regard to the pandemic, what do you hope your project can achieve by May 2021 (the end of the 4th year budget period for CIFASD4) and/or what COVID-19 impacts will remain obstacles?*

We anticipate that if nothing changes with regard to the pandemic in the next 6 months, we will proceed in the first quarter of 2021 to do the preliminary analysis of the school-age testing results on the sample of 96 children. We will also complete analyses of preschool data. We anticipate a blood sample shipment from samples collected earlier in 2020 to arrive in San Diego by December, 2020. With these samples, Drs. Weinberg and Miranda will do additional analysis of maternal samples and paired samples from school age children (as appropriate).

Administrative Supplements. *For this reporting period (June 2020 to present), please list any Administrative Supplement associated with this award and a sentence or two about what it entails.*

One supplement covers two objectives. One is to determine the prevalence of the co-occurrence of substantial prenatal alcohol exposure in pregnant women who are infected with HIV, and if there is a sufficient prevalence of co-exposure, could this group be evaluated for child neurodevelopment. The second is to determine if phosphatidyl ethanol biomarker in maternal blood collected around the time of enrollment and in the third trimester validates maternal report of alcohol use.

For the first aim, the preliminary work was completed in the Volyn Province of Ukraine through the provincial HIV clinic where pregnant women and their children receive services. A total of 101 women who were HIV positive during their pregnancies with their 112 18-month old to 10-year-old children were interviewed about alcohol exposure in pregnancy. As shown in the Table, prenatal exposure to alcohol and frequent drinking is common in this sample, and children were 2-4 times more likely to be born small with small head circumference than the general population in other provinces in Ukraine. Subjective maternal concerns about their child's development was most commonly about behavior.

Characteristics	**Children N =112**	**Population Reference in Ukraine**
Male	63 (56.3%)	
Female	49 (43.8%)	
Birthweight <2500 g	12 (10.7%)	4.3%
OFC at birth <32 cm	16 (14.3%)	3.3%-4.1%
Maternal alcohol consumption first month of pregnancy any 3 to 4 occasions	 40 (36%) 18 (16%)	
Maternal concerns about development Learning Behavior	 7 (6.3%) 14 (12.5%)	

For the second aim, 39 samples from 32 women were analyzed by USDTL. A second set of maternal blood samples is expected to be received in December, 2020, and sent to USDTL. Concordance for samples collected near the time of maternal interview was excellent for those who were exposed by maternal report, and discordant in a small portion of those who were unexposed by maternal report.

Publications June 2017 - present

Publications [Accepted & In Press]

Bandoli G, Jones K, Wertelecki W, Yevtushok L, Zymak-Zakutnya N, Granovska I, Plotka L, Chambers C; CIFASD. Patterns of prenatal alcohol exposure and alcohol-related dysmorphic features. *Alcohol Clin Exp Res.* 2020 Aug 9; In press.

Salem NA, Mahnke AH, Wells AB, Tseng AM, Yevtushok L, Zymak-Zakutnya N, Wertlecki W, Chambers CD, Miranda RC; CIFASD. Association between fetal sex and maternal plasma microRNA responses to prenatal alcohol exposure: evidence from a birth outcome-stratified cohort. *Biol Sex Differ*. 2020 Sep 10;11(1):51. doi: 10.1186/s13293-020-00327-2. PMID: 32912312 PMCID: PMC7488011

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Bandoli G, Coles CD, Kable JA, Wertelecki W, Yevtushok L, Zymak-Zakutnya N, Wells A, Granovska IV, Pashtepa AO, Chambers CD; CIFASD. Patterns of prenatal alcohol use that predict infant growth and development. *Pediatrics*. 2019 Feb;143(2):e20182399. doi: 10.1542/peds.2018-2399. Epub 2019 Jan 4. PMID: 30610099 PMCID: PMC6361345

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; CIFASD. Altered maternal immune networks are associated with adverse child neurodevelopment: Impact of alcohol consumption during pregnancy. *Brain Behav Immun*. 2018 Oct;73:205-215. doi: 10.1016/j.bbi.2018.05.004. Epub 2018 May 5. PMID: 29738852 PMCID: PMC6344127

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Publications [In Preparation & Submitted]

Kautz C, Petrenko CLM, Handley ED, Coles CD, Kable JA, Wertelecki W, Yevtushok L, Zymak-Zakutnya N, Chambers CD, and CIFASD. Partner influence as a factor in maternal alcohol consumption and depressive symptoms, with subsequent effects on infant neurodevelopmental outcomes. Submitted to *ACER*.

Kable JA, Coles CD, Jones KL, Yevtushok L, Kulikovsky Y, Zymak-Zakutnya N, Dubchak I, Adhmedzhanova D, Wertelecki W, Chambers CD, and the CIFASD. Infant cardiac orienting responses predict later FASD in the preschool period. Submitted to *ACER*.

Coleman TP, Kable JA, Aguilar-Rivera M, Coles CD, Jones KL, Yevtushok L, Kulikovsky Y, Zymak-Zakutnya N, Akhmedzhanova D, Wertelecki W, Chambers CD. Infant cardiac orienting response as a predictor of neurodevelopmental risk. Submitted.

Raineki C, Bodnar T, Wertelecki W, Yevtushok L, Plotka L, Granovska I, Zymak-Zakutnya N, Pashtepa A, Wells A, Honerkamp-Smith G, Coles CD, Kable JA, Chambers CD, Weinberg J, and the CIFASD. Differential associations between maternal and child Immune milieus in alcohol-dependent and alcohol-independent neurodevelopmental delay. In preparation.

Principal Investigator: Jeffrey R. Wozniak
Institution: University of Minnesota
CIFASD4 Project: Multi-Modal Connectivity Methods for the Validation of FASD Diagnostic Criteria
Grant Number: 5U01AA026102-04

Specific Aims

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.

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.

Accomplishments

1) **Major Activities.** Despite COVID-19, we have resumed some research visits with neuropsychological testing and MRI scans for our longitudinal participants. Total participants enrolled = 101 (49 PAE and 52 control). These numbers represent 109% and 116% of our targets, respectively. For our second visits (the longitudinal portion of the study), we have so far scanned and evaluated 42 participants (26 PAE and 16 Control). These numbers represent 130% and 80% of our May, 2021 goal (we are ahead of schedule).

   - All data are being processed as they are acquired. We are continuing to publish CIFASD data.
   - Acquisition of 3D facial images for Dr. Michael Suttie's project = 101.
   - Blood samples (n=67) obtained from Dr. Wozniak's R01 project (R01AA024123) have been sent to Dr. Joanne Weinberg's lab at UBC for immune function studies.
   - Neurocognitive data continue to be shared with Dr. Sarah Mattson for her Decision Tree project.
   - We are also gearing up to have our participants take Dr. Mattson's remotely-administered neuropsychological tests.

2) Specific Objectives. For the coming months, we plan to continue to see participants for our second visits to keep the longitudinal gap as close to 15 months as possible, despite the pandemic which has disrupted scheduling to some extent.

3) Significant Results.

- We have found unique associations between structural volumes in components of the "social brain" and behaviors reflective of social functioning (paper under review).
- We identified sub-fields within the hippocampus that are abnormal in children with PAE compared to controls (paper in press).
- We identified para-limbic structures that are atypical in children with PAE compared to controls and investigated relationships with internalizing and externalizing symptoms (anxiety, depression, and other behavioral issues) (paper accepted).
- We have investigated cortical myelin in our sample and found no differences between PAE and controls. This negative finding is novel and important and we have a manuscript in preparation on these data.

4) Key Outcomes and Other Achievements. We are well-ahead of schedule for targets for MRI scans and neurocognitive evaluations.

Additional Questions

CIFASD4 Synergy. *Describe your project's interrelation with aims of the CIFASD consortium its other projects and the progress on those interactions.*

- All other PIs: sharing of brain imaging data via the central repository
- Weinberg: sharing of blood samples for her project
- Mattson: sharing of neuropsychological data both ways
- Suttie: sharing of 3D facial images
- Jones: sharing of dysmorphology data
- Foroud: sharing of saliva samples for genetic analyses and referrals being made to the DIGS study
- Petrenko: referring participants for her focus groups, etc.

Pandemic - May 2021. *If nothing changes with regard to the pandemic, what do you hope your project can achieve by May 2021 (the end of the 4th year budget period for CIFASD4) and/or what COVID-19 impacts will remain obstacles?*

COVID-19 is definitely posing serious challenges, but we are currently allowed to run participants in our longitudinal study and we are taking advantage of the window of opportunity to complete as many MRI scans and neurocognitive assessments as possible. By May, 2021, we will be ahead of schedule on these longitudinal procedures.

Administrative Supplements. *For this reporting period (June 2020 to present), please list any Administrative Supplement associated with this award and a sentence or two about what it entails*. None

Publications/Abstracts June 2017 - present

Publications [Accepted & In Press]

Roediger, D.J., Krueger, A.M., de Water, E., Mueller, B.A., Boys, C.A., Hendrickson, T.J., Schumacher, M.J., Mattson, S.N., Jones, K.L., Lim, K.O., CIFASD, & **Wozniak, J.R.** (In press). Hippocampal subfield abnormalities and memory functioning in children with fetal alcohol spectrum disorders. *Neurotoxicology & Teratology*.

Krueger, A., Roediger, D., Mueller, B.A., Boys, C.J., Hendrickson, T.J., Schumacher, M.J., Mattson, S.N., Jones, K.L., Riley, E.P., Lim, K.O., CIFASD, and **Wozniak, J.R.** (2020). Para-limbic structural abnormalities are associated with internalizing symptoms in children with prenatal alcohol exposure. *Alcoholism: Clinical and Experimental Research; 44(8):1598-1608.* PMCID: PMC784415.

Uban, K.A., Kan, E., **Wozniak, J.R.**, Mattson, S.N., Coles, C., & Sowell, E.R. (2020). The relationship between socioeconomic status and brain volume is attenuated in children and adolescents with prenatal alcohol exposure. *Frontiers in Human Neuroscience; 14(85).* PMCID: PMC7156853.

Doyle, L.R., Glass, L., **Wozniak, J.R.,** Kable, J.A., Riley, E.P., Coles, C.C., Sowell, E.R., Jones, K.L., Mattson, S.N., and the CIFASD (2019). Relation between oppositional/conduct behaviors and executive function among youth with histories of heavy prenatal alcohol exposure. *Alcoholism: Clinical and Experimental Research; 43(6):1135-1144.* PMCID: PMC6551300.

Doyle, L.R., Moore, E.M., Coles, C.C., Kable, J.A., Sowell, E.R., **Wozniak, J.R.**, Jones, K.L., Riley, E.P., Mattson, S.N., and the CIFASD (2018). Executive functioning correlates with communication ability in youth with histories of heavy prenatal alcohol exposure. *Journal of the International Neuropsychological Society, 24(10):1026-1037*. PMCID: PMC6237635.

Suttie, M.M., **Wozniak, J.R.**, Wetherill, L.M., Mattson, S.M., Sowell, E.R., Kan, E., Riley, E.P., Jones, K.L., Coles, C.C., Foroud, T., Hammond, P., & the CIFASD (2018). Combined face-brain morphology and associated neurocognitive correlates in Fetal Alcohol Spectrum Disorders. *Alcoholism: Clinical and Experimental Research; 42(9):1769-1782.* PMCID: PMC6120799.

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

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Gross, L.A., Moore, E.M., Coles, C.D., Kable, J.A., Sowell, E.R., **Wozniak, J.R.**, Jones, K.L., Riley, E.P., Mattson, S.N., & the CIFASD. (2017). Neural correlates of verbal memory in youth with heavy prenatal alcohol exposure. *Brain Imaging and Behavior, 12(3): 806–822.* PMCID: PMC5745322.

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Publications [In Preparation & Submitted]

De Water, E., Rockhold, M.N., Roediger, D.J., Krueger, A.M., Mueller, B.A., Boys, C.J., Schumacher, M.J., Mattson, S.N., Jones, K.L., Lim, K.O., CIFASD, & **Wozniak, J.R.** (under review). Social behaviors and gray matter volumes of brain areas supporting social cognition in children and adolescents with prenatal alcohol exposure.

Poster Abstracts and Presentations

Rockhold, M.N., Krueger, A.M., Schumacher, M.J., Mattson, S.N., Jones, K.L., Riley, E.P., & **Wozniak, J.R.** (2021, February*). The association of ADHD symptoms and learning ability in children with prenatal alcohol exposure*. Poster to be presented at the International Neuropsychological Society Conference, San Diego, CA.

De Water, Krueger, A.M., E. Rockhold, M.N., Roediger, D.J., Mueller, B.A., Boys, C.A., Hendrickson, T.J., Schumacher, M.J., Mattson, S.N., Jones, K.L., Riley, E.P., Lim, K.O., & **Wozniak, J.R.** (2021, February*). Social Behaviors and gray matter volumes of brain areas supporting social cognition in children and adolescents with prenatal alcohol exposure*. Poster to be presented at the International Neuropsychological Society Conference, San Diego, CA.

Krueger, A.M., Rockhold, M.N., Roediger, D.J., Mueller, B.A., Boys, C.A., Hendrickson, T.J., Schumacher, M.J., Mattson, S.N., Jones, K.L., Riley, E.P., Lim, K.O., & **Wozniak, J.R.** (2020, June*). Para-limbic structural abnormalities' association with internalizing symptoms in children with prenatal alcohol exposure.* Poster presented at the Research Society on Alcoholism Annual Scientific Meeting, New Orleans, LA.

de Water, E., Krueger, A.M., Lindgren, C.W., Fuglestad, A.J., Rockhold, M.N., Sandness, K.E., Eckerle, J.K., Fink, B.A., Boys, C.J., and **Wozniak, J.R.** (2020, June). *Early delay of gratification predicts later inhibitory control and academic performance in children with prenatal alcohol exposure*. Poster presented at the Research Society on Alcoholism Conference, New Orleans, Louisiana.

Rockhold, M.N., Krueger, A.M., Schumacher, M.J., Boys, C.J., Mattson, S.N., Riley, E.P., Jones, K.L., **Wozniak, J.R.** (2020, June). *Social behavior and executive functioning deficits in children prenatally exposed to alcohol*. Research Society on Alcohol Conference, New Orleans, LA.

Glass, L., Moore, E.M., Coles, C.D., Kable, J.A., Adnams, C. May, C., Sowell, E.R., **Wozniak, J.R.**, Riley, E.P., Mattson, S.N., and the CIFASD. (2020, February). *Differential relations between adaptive behavior and age for youth with prenatal alcohol exposure.* Poster presented at the International Neuropsychological Society Conference, Denver, Colorado.

Wozniak, J.R. (2019, August). *Fetal Alcohol Spectrums Disorder & FASD in the context of child development, education, and independence.* All-day workshop presented with the University of Nebraska College of Public Health, Department of Health Promotion, Oglala Lakota County School District, Pine Ridge Indian Reservation, South Dakota.

Wozniak, J.R. (2019, August). *Fetal Alcohol Spectrum Disorders: Half-day workshop.* Workshop presented with the University of Nebraska College of Public Health, Department of Health Promotion, Gordon-Rushville High School, Gordon, Nebraska.

Wozniak, J.R. (2018, November). *FASD research update: Brain imaging and the development of smart interventions*. Keynote address at the 7th Annual FASD Matters Conference, Brooklyn Park, Minnesota.

Wozniak, J.R. (2018, April). *Brain sciences and FASD: Imaging and neuropsychology.* Invited plenary talk as part of the *"Let's Talk – Evidence, Experience, and Wisdom in the Room series for families,* at the 8th International Conference on Adolescents and Adults with FASD, Vancouver, British Columbia, Canada.

Doyle, L.R., Glass, L., Coles, C.D., Kable, J.A., Sowell, E.R., **Wozniak, J.R.**, Riley, E.P., Mattson, S.N., and the CIFASD (2018, February). *Impact of comorbid oppositional behavior on executive functioning among youth with histories of heavy prenatal alcohol exposure*. Poster given at the annual meeting of the International Neuropsychological Society, Washington D.C.

Wozniak, J.R. (2017, April). *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.

Principal Investigator: Sarah Mattson
Institution: San Diego State University
CIFASD4 Project Title: Multisite Neurobehavioral Assessment of FASD
Grant Number: 5U01AA014834-17

Specific Aims

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 existing data from lower risk samples.**
    b. **Explore feasibility, sensitivity, and specificity of the CIFASD Decision Tree in clinical settings using an internet-based or mobile app version of the CIFASD Decision Tree for identification of children affected by prenatal alcohol exposure. Results of the CIFASD Decision Tree will be validated using advanced neuropsychological data.**
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.**
    b. **Validate online neurobehavioral tool (FONS) in a subset of subjects.**

Accomplishments

Our major activities, objectives, significant results and achievements fall under four main projects, guided by our specific aims. These are archival data analysis, decision tree (eTree), validation test battery, and the BRAIN-online (formerly FONS). Activities and achievements are described in the following sections.

1) Major Activities.

Archival Data Analysis (Aim 1a): As reported in previous progress reports, we examined the classification accuracy of the eTree app in the San Diego coFASP data set (Chambers, PI). Overall accuracy was 83% but subgroup accuracy varied from 28% to 100%. We are currently examining the role of moderator and mediator variables in this analysis and considering whether alterations to the eTree algorithm would improve accuracy in low-risk samples. We have also obtained data from the other coFASP sites (May, PI) and plan to use that dataset to test the algorithm modifications.

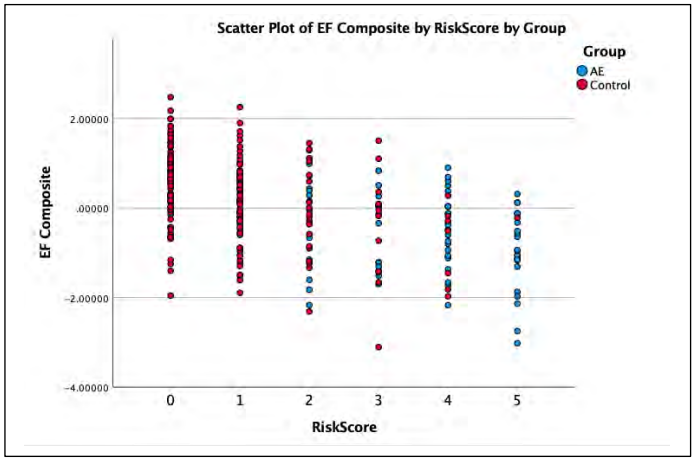
eTree (Aim 1b): We continue to collect data using the eTree. This has been substantially slowed due to the pandemic; however, our current completed sample is 269, which is 87% of our May 2021 goal. Of these subjects, 209 are from San Diego and 60 are from Minneapolis. We are still enrolling subjects that are seen in person in the UCSD clinic (K. Jones) but this has slowed considerably during the pandemic.

Validation of the eTree using in-person neuropsychological testing (Aim 1b): Due to the COVID-19 pandemic, all in-person testing has paused. Our current completed sample is 215, which is 115% of our May 2021 goal. Of these subjects, 114 are from San Diego and 101 are from Minnesota.

BRAIN-online (Aim 2a/2b): Since the last progress report, we have rolled out the Brief Assessment of Individual Neurobehavior – online version (BRAIN-online) which was formerly known as the FONS. We enrolled our first subject on 9/7/20 and have tested 38 subjects so far. This represents 76% of the subjects who were sent invitations. The subjects are 7-17 years old, with an average age of 12.25 years. We started our recruitment for BRAIN-online with subjects that already had eTree and in-person neuropsychological testing data and thus 95% of the BRAIN-online sample also have eTree and neuropsychological test data.

2) Specific Objectives. The objectives of this project remain to test subjects using existing measures, develop and implement novel online tests, and validate both the eTree and BRAIN-online using in-person neuropsychological testing.

3) Significant Results. We have completed or are near completion of two studies. In the first study, we report the development and validation of the risk score that is included in the eTree. This risk score was developed using the same measures included in the decision tree except for IQ score which was used to validate the risk score. The risk score was developed using data from CIFASD 2 (N=325) and validated using data from CIFASD 3 (N=426). In the development cohort, the resulting risk score, which ranged from 0-5 could be used to distinguished subjects with prenatal alcohol exposure from controls. Frequencies of the Alcohol Exposed (AE) and control (CON) subjects in each risk group were significantly different (χ^2 = 81.97, p <.0001). Subjects in the "high risk" category (risk score of 4-5) were more likely to be in the AE group compared to those in the "low risk" group (risk score of 0-1; *Odds Ratio [OR]* = 29.37, p <.0001, 95% CI: 13.74-62.79). The risk score was also significantly correlated with an executive function composite score in the AE group (r = -.300, p = .002; see figure) and CON group (r = -.403, p <.0001) and with IQ score for both groups (AE: r = -.349, p <.001; CON: r = -.313, p <.0001).



In the second study, we compared ratings of executive function from multiple informants (self, parent, teacher) to laboratory measures. The data analyzed were part of CIFASD 2 and included subjects with histories of prenatal alcohol exposure (AE; n = 47), attention-deficit/hyperactivity disorder (ADHD; n = 41), and typically developing controls (CON; n = 73). Weak correlations were found between rating scales and laboratory measures, indicating poor convergent validity for the behavior rating scale. Inter-rater reliability was found but differed by group. Agreement was found between parent and teacher ratings for children with prenatal alcohol exposure, whereas teacher-child agreement was found for those with ADHD. Findings from this study indicate that behavior ratings can be used to supplement laboratory measures but may not be measuring cognitive abilities regardless of whether a clinical diagnosis is present. A multi-method approach should be used when measuring skills in this domain. This was one of the first studies to examine cross-informant agreement in a sample of children with prenatal alcohol exposure. This paper was submitted to ACER and is currently being revised for resubmission.

We are also continuing our efforts on 3 papers aimed at validating the eTree. These have been described previously and won't be repeated here given space limitations.

4) Key Outcomes and Other Achievements. Our most significant achievement since the last progress report was the development and deployment of BRAIN-online. We have tested 38 subjects so far and preliminary data are promising. We are currently in the early stages of four other significant projects. The first two are designed to collect normative data on BRAIN-online. We plan to recruit school-age and young adult samples using an entirely online recruitment and testing protocol. We are preparing the IRB and test materials for these projects. The second two projects involve data collected in CIFASD 4 and previous phases. The first of these is consistent with Aims 1b (validation of the eTree). We are examining the relationship between three parent questionnaires: CBCL, VABS, and BASC. The first two are part of the eTree and have been used in multiple studies from our lab. The BASC is another parent questionnaire that incorporates both problem behaviors and adaptive function. In our goal to make the eTree as broadly useful, we want to test whether the BASC is a viable substitute to the current measures (CBCL and VABS). We are just beginning the analyses on this project. The second planned project is an examination of how co-occurring maternal alcohol and other substance use (e.g., opioids, cannabis) during pregnancy impacts neurodevelopment and neurobehavior in children. These analyses are also in the planning stage and will include existing CIFASD data as well as data from other projects in my lab.

Additional Questions

CIFASD4 Synergy. *Describe your project's interrelation with aims of the CIFASD consortium its other projects and the progress on those interactions.*

This project involves direct collaborations with several other U01 projects, including those directed by Drs. Foroud, Suttie, Chambers, and Wozniak. Close collaboration between the Dysmorphology Research Resource is critical for the success of the project. Further, the Administrative Resource provides support for programming and web design needed for the success of the project. Finally, the Informatics Research Resource supports database development and data storage and sharing.

Pandemic - May 2021. *If nothing changes with regard to the pandemic, what do you hope your project can achieve by May 2021 (the end of the 4th year budget period for CIFASD4) and/or what Covid-19 impacts will remain obstacles?*

While this project is fortunate to rely heavily on electronic data collection, much of which can be continued during the pandemic, there are aspects that are directly affected by the pandemic. Recruitment of new subjects has slowed dramatically and in person testing was paused in March of 2020. If nothing changes with regard to the pandemic by May 2021, we anticipate being able to complete most of our aims and objectives. The one exception is that we have not been able to recruit subjects from the developmental - behavioral pediatrics clinic, which was proposed as part this project. In addition, we have subjects that were recruited for the eTree from Dr. Wozniak's project. They have been tested but have not yet been examined by Dr. Jones. Otherwise, we will be able to continue to meet our study aims – developing, deploying and validating the eTree and developing, deploying, and validating BRAIN online. Continued pandemic-related restrictions will affect recruitment of new subjects and completion of others, as described above.

Administrative Supplements

3U01AA014834-16S1. This is a "Research Supplement to Promote Diversity in Health-Related Research" and supports Carissa Zambrano.

Publications/Abstracts June 2017 - Present

Publications [Accepted & In Press]

Peer-Reviewed Papers

Roediger, D.J., Krueger, A.M., de Water, E., Mueller, B.A., Boys, C.A., Hendrickson, T.J., Schumacher, M.J., **Mattson, S.N.**, Jones, K.L., Lim, K.O., CIFASD, Wozniak, J.R. (2020 in press). Hippocampal subfield abnormalities and memory functioning in children with fetal alcohol spectrum disorders. Neurotoxicology and Teratology, in press. doi: 10.1016/j.ntt.2020.106944

Moore, E.M., Glass, L., Infante, A., Coles, C.D., Kable, J.A., Jones, K.L., Riley, E.P., **Mattson, S.N.**, and the CIFASD (2020). Cross-sectional analysis of spatial working memory development in children with histories of heavy prenatal alcohol exposure. Alcoholism: Clinical and Experimental Research, in press. doi: 10.1111/acer.14506

Krueger, A.M. Roediger, D.J., Mueller, B.A., Boys, C.A., Hendrickson, T.J., Schumacher, M.J., **Mattson, S.N.**, Jones, K.L., Riley, E.P., Lim, K.O., Wozniak, J.R. (2020 in press). Para-limbic structural abnormalities are associated with internalizing symptomology in children with prenatal alcohol exposure. Alcoholism: Clinical and Experimental Research, in press. doi: 10.1111/acer.14390 PMCID: PMC7484415

Uban, K.A., Kan, E., Wozniak, J., **Mattson, S.**, Coles, C.D., Sowell, E.R., & the CIFASD (2020, in press). The relationship between socioeconomic status and brain development is attenuated in children and adolescents with prenatal alcohol exposure. Frontiers in Human Neuroscience, Available online 08 April 2020. doi: 10.3389/fnhum.2020.00085 PMCID: PMC7156853https://pubmed.ncbi.nlm.nih.gov/32322193/

Simmons, R.W., Taggart, T.C., Thomas, J.D., **Mattson, S.N.**, Riley, E.P. (2020). Gait control in children with attention-deficit/hyperactivity disorder. Human Movement Science, 70, 102584. Available online 8 February 2020 doi: 10.1016/j.humov.2020.102584 PMCID: In Progress (NIHMSID: 159672)

Kable, J.A., Coles, C.D., **Mattson, S.N**. and the CIFASD (2020). Neurodevelopmental outcomes associated with prefrontal cortical deoxygenation in children with fetal alcohol spectrum disorders. Developmental

Neuropsychology, 45 (1): 1-16. Available online 8 January 2020 doi: 10.1080/87565641.2020.1712604 PMCID: PMC7080191

Doyle*, L.R., Glass, L., Wozniak, J.R., Kable, J.A., Riley, E.P., Coles, C.D., Sowell, E.R., Jones, K.L., **Mattson, S.N.** and the CIFASD (2019). Relation between oppositional/conduct behaviors and executive function among youth with histories of heavy prenatal alcohol exposure. Alcoholism: Clinical and Experimental Research, 43 (6): 1135-1144. doi: 10.1111/acer.14036 PMCID: PMC6551300

Mattson, S.N., Bernes*, G.A, Doyle*, L.R. (2019). Fetal alcohol spectrum disorders: A review of the neurobehavioral deficits associated with prenatal alcohol exposure. Alcoholism: Clinical and Experimental Research, 43 (6): 1046-1062. Available online 2 May 2019.doi: 10.1111/acer.14040 PMCID: PMC6551289 This paper was the most downloaded article from the journal in 2019.

Doyle*, L.R., Coles, C.D., Kable, J.A., May, P.A., Sowell, E.R., Jones, K.L., Riley, E.P., **Mattson, S.N.**, and the CIFASD (2019). Relation between adaptive function and IQ among youth with histories of heavy prenatal alcohol exposure. Special issue on Fetal Alcohol Spectrum Disorders, Birth Defects Research, 111 (12): 812-821. doi: 10.1002/bdr2.1463 PMCID: PMC6650363

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

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

Seewald, P.M, DeJesus, S.Y., Graves, L.V., Moreno, C.C., **Mattson, S.N.** and Gilbert, P.E. (2018). Age-related differences on a new test of temporal order memory for everyday events. Aging, Neuropsychology and Cognition, 25 (3): 319-332. Available online 07 March 2017 doi: 10.1080/13825585.2017.1298716 PMCID: PMC5935107

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

Doyle*, L.R., Moore, E.M., Coles, C.D., Kable, J.A., Sowell, E.R., Wozniak, J.R., Jones, K.L., Riley, E.P. **Mattson, S.N.** and the CIFASD (2018). Executive functioning correlates with communication ability in youth with histories of heavy prenatal alcohol exposure. Journal of the International Neuropsychological Society, 24 (10): 1026-1037. doi: 10.1017/S1355617718000772 PMCID: PMC6237635

Glass*, L., Moore, E.M., Akshoomoff, N., Jones, K.L., Riley, E.P., **Mattson, S.N.** (2017). Academic difficulties in children with prenatal alcohol exposure: Presence, profile, and neural correlates. Alcoholism: Clinical and Experimental Research, 41 (5), 1024-1034. Available online 24 Mar 2017. doi: 10.1111/acer.13366 PMCID: PMC5404947

Infante, M.A., Moore, E.M., Bischoff-Grethe, A., Tapert, S.F., **Mattson, S.N.**, and Riley, E.P. (2017). Altered functional connectivity during spatial working memory in children with heavy prenatal alcohol exposure. Alcohol, 64, 11-21. Available online 2017 Aug 12.doi: 10.1016/j.alcohol.2017.05.002. PMCID: PMC5635832

Book Chapters

Bernes*, G., Moore, E., Vaurio, L., and **Mattson, S.N.** (2020, in press). Fetal alcohol spectrum disorders. In M. Beauchamp, R. Peterson, M.D. Ris, H.G. Taylor, and K. Yeates, (Eds.), Pediatric Neuropsychology: Research, Theory, and Practice, 3rd Edition.New York, NY: Guilford Press.

Mattson, S.N., Doyle*, L.R., Glass*, L. (2020, in press). Fetal Alcohol Spectrum Disorders and Other Teratogenic Conditions. To appear in the APA Handbook of Intellectual and Developmental Disabilities.

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

Publications [In Preparation & Submitted]

Submitted

Rockhold, M. Krueger, A., de Water, E., Lindgren, C., Sandness, K., Eckerle, J., Schumacher, M., Fink, B., Boys, C., Carlson, S., Fuglestad, A., **Mattson, S.N.**, Jones, K.; Riley, E., Wozniak, J. (Submitted 2020). Executive and Social Functioning Across Development in Children and Adolescents with Prenatal Alcohol Exposure.

Bernes*, G.A., Coles, C.D., Kable, J.A., May PA., Kalberg, W.O., Sowell, E.R., Jones, K L., Riley, E.P., **Mattson, S.N.**, and the CIFASD (Submitted 2020). Convergent validity of measures of executive function in children with heavy prenatal alcohol exposure: Correspondence between multiple raters and laboratory measures.

De Water, E., Rockhold, M, Roediger, D., Krueger, A., Mueller, B., Boys, C., Schumacher, M., **Mattson, S.N.**, Jones, K.L., Lim, K., Wozniak, J.R. (Submitted 2020). Social Behaviors and Gray Matter Volumes of Brain Areas Supporting Social Cognition in Children and Adolescents with Prenatal Alcohol Exposure.

In Preparation

Bernes, Courchesne, Mattson et al., Development of a Postnatal Risk Score that Identifies Children with Prenatal Alcohol Exposure

Mattson, Duprey, Hyland, et al. Sensitivity and Specificity of an automated decision tree tool for detecting FASD.

Hyland, Duprey, E.E., Chambers, C.D., Mattson, S.N., et al. Validation of an automated decision tree tool for detecting FASD in a low-risk prevalence sample.

Duprey, Hyland, Jones, Mattson et al., Accuracy of an automated decision tree tool for detecting ARND.

Poster Abstracts and Presentations (2020)

Mattson, S.N., Duprey, E.E., Hyland, M.T., Wozniak, J.R., Jones, K.L., Chockalingam, G., Riley, E.P., and the CIFASD (2020). Sensitivity and Specificity of an automated decision tree tool for detecting FASD. To be Presented at the Research Society on Alcoholism meeting, New Orleans, June 2020. (Conference Cancelled).

Hyland, M.T., Duprey, E.E., Chambers, C.D., **Mattson, S.N.**, and the CIFASD (2020). Validation of an automated decision tree tool for detecting FASD in a low-risk prevalence sample. To be Presented at the Research Society on Alcoholism meeting, New Orleans, June 2020. (Conference Cancelled).

Duprey, E.E., Hyland, M.T., Jones, K.L., **Mattson, S.N.**, and the CIFASD (2020). Accuracy of an automated decision tree tool for detecting ARND. To be Presented at the Research Society on Alcoholism meeting, New Orleans, June 2020. (Conference Cancelled).

Glass, L., Moore, E.M., Coles, C.D., Kable, J.A., May, P.A., Kalberg, W.O., Adnams, C. Sowell, E.R., Wozniak, J.R., Riley, E.P., **Mattson, S.N.**, and the CIFASD (2020). Differential relations between adaptive behavior and age for youth with prenatal alcohol exposure. Presented at the International Neuropsychological Society Meeting, Denver, February 2020.

Principal Investigators: Scott Parnell; Johann Eberhart
Institutions: University of North Carolina; University of Texas
CIFASD4 Project: Exploring the Genetics of FASD in Complementary Mouse and Fish Models
Grant Number: 5U01AA021651-09

Specific Aims

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.

Accomplishments

1) Major Activities. We have finished up analyses on our p53, Bax, and initial 6J vs 6N RNA-Seq studies, and all three of these manuscripts are in the process of being written, with the p53 and RNA-Seq manuscripts in the final editing stages. The Bax wild-type vs KO RNA-Seq is completed and currently being analyzed. We have continued progress on our analyses of genes implicated in humans, Htt and Kif2a. Our Efcab7 studies in both mouse and zebrafish continue. We are finalizing our analyses of zebrafish nnt mutants, demonstrating that they are ethanol sensitive. We have resumed our forward genetic screen and have switched to a transposon-based approach that should speed gene discovery. Finally, we have begun the initial project planning for continuing these genetic studies in the collaborative cross, which, when completed will provide a wealth of transcriptomic and genomic data for further interrogation in mice, zebrafish, and humans.

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. Given that Nnt is known to have a mutation in the ethanol sensitive 6J strain, our zebrafish findings suggest that this mutation may be a significant cause of ethanol sensitivity in this strain. Knocking out Htt in mouse seems to be protective, but our initial analyses suggest this phenomenon may

only be manifested in female offspring. However, these data are still very preliminary. Bax RNA-Seq analysis reveals about 60 genes are differentially expressed between the normal, susceptible wild-types and the KOs, which are completely protected against ethanol.

4) Key Outcomes and Other Achievements. Finished our 6J vs. 6N analysis and are almost ready for publication, which not only will detail the precise differences between these strains, it will tell us the key transcriptomic changes following ethanol exposure, as well provide a database of genes that are being expressed in the developing embryo during gastrulation. These data are currently being incorporated into a searchable website-based database that will hopefully prove extremely useful to not just FAS researchers, but many people in the developmental biology field.

Additional Questions

CIFASD4 Synergy. *Describe your project's interrelation with aims of the CIFASD consortium its other projects and the progress on those interactions.*

We have been working with the human genetics project (Foroud/Wetherill), particularly with our Htt and Kif2a experiments, but also suggesting additional genes that they can probe in their human datasets.

Pandemic - May 2021. *If nothing changes with regard to the pandemic, what do you hope your project can achieve by May 2021 (the end of the 4th year budget period for CIFASD4) and/or what COVID-19 impacts will remain obstacles?*

We think we can publish the manuscripts currently in preparation, finish the Htt, Efcab7, and Nnt experiments, and begin stage 1 of the collaborative cross experiment.

Administrative Supplements. *For this reporting period (June 2020 to present), please list any Administrative Supplement associated with this award and a sentence or two about what it entails.* n/a

Publications June 2017 - present

Publications [Accepted & In Press]

Everson J.L., Batchu R. and Eberhart J.K. Multifactorial genetic and environmental hedgehog pathway disruption sensitizes embryos to alcohol-induced craniofacial defects. *Alcohol Clin Exp Res*, DOI: 10.1111/acer.14427, 2020, In press.

Boschen KE, Ptacek TS, Simon JM, Parnell SE. Transcriptome-wide regulation of key developmental pathways in the mouse neural tube by prenatal alcohol exposure. *Alcohol Clin Exp Res*, 44:1540-1550, 2020. PMCID: PMC7484470

Swartz M.E., Lovely C.B., McCarthy N., Kuka T., Eberhart J.K. Novel ethanol-sensitive mutants identified in an F3 forward genetic screen. *Alcohol Clin Exp Res*, 44, 56-65, 2020. PMCID: PMC6980918

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Buckley D., Sidik A., Kar R.D. and Eberhart J.K. Differentially sensitive neuronal subpopulations in the central nervous system and the formation of hindbrain heterotopias in ethanol-exposed zebrafish. *Birth Defects Research,* 111, 700-713, 2019. PMCID: PMC6650308

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Publications [In Preparation & Submitted]

Sidik A, Dixon G, Kirby HG, and Eberhart JK. Gene-environment interactions characterized by single embryo transcriptomics (in revision, *BMC Biology*)

Fish EW, Tucker SK, Peterson RL, Eberhart JK, Parnell SE. Tp53 is a pathogenic mechanism for gastrulation stage alcohol exposure: studies in mice and zebrafish (*in prep*)

Boschen KE, Ptacek KS, Simon JM, Parnell SE Genetic variation that underlies differences in prenatal alcohol sensitivity in mice. (*in prep*)

Mazumdar R., Parnell SE and Eberhart J.K. Nnt function is required to protect against ethanol teratogenesis. (*in prep*)

Kuka T. and Eberhart J.K. A novel LRP family member protects against multiple environmental insults. (*in prep)*

Principal Investigators: Christie Petrenko; Cristiano Tapparello
Institution: University of Rochester
CIFASD4 Project: Development and Evaluation of an Evidence-Based Mobile Health Caregiver Intervention for FASD
Grant Number: 5U01AA026104-04

Specific Aims

The goal of this project is to systematically develop and evaluate an evidence-based mobile health (mHealth) intervention for caregivers raising children with FASD. This mHealth intervention is designed to be scalable and reduce barriers to care. Work is proceeding with the following aims:

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 Families Moving Forward (FMF) Program for caregivers raising children with FASD, and building on our existing framework for the development of medical apps.

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 60 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.

Accomplishments

1) Major Activities.

Final analyses are nearly complete for the two rounds of beta-testing completed as part of **Aim 1** (April-May 2019; November-December 2019). Focus group and interview data were available for 26 caregivers and 16 providers who tested the app. Systematic thematic coding of transcripts was initiated in May 2020 and is expected to be complete at the start of December 2020. Our team selected a combination of Evaluative, Values, and Structural coding methods to answer our primary research objectives. The initial codebook was established through consensus across 4 independent coders and regular reliability meetings continue weekly. See the results section below for an updated summary of our current analytic model. A manuscript is currently in preparation for this data with a goal of submission in January 2021.

Aim 2 is well underway. Recruitment for the Feasibility Trial began at the end of January 2020. Baseline data collection was initiated with iOS users at the end of February, with the app release starting in mid-March. Quantitative and qualitative follow-up data collection for iOS users was largely completed between June – September 2020. See the results section for initial metrics.

Programming has continued on the Android version of the app through the summer and early fall. Approximately 60% of the code had to be re-written due to changes with Android OS, Amazon Web Services, and inconsistencies discovered on testing. New features were also added to align it with the iOS version. Furthermore, based on user data and feedback from beta-test 2 and iOS feasibility users, we have decided to unlock all content in the Android version for users from the start. This will provide users more autonomy in self-directing their learning. We will test whether Learning Module completion and usage increases relative to prior tests following this change, which will inform refinements to the app for the RCT planned in 2021. Baseline data collection for Android will start within the next few weeks.

Our team has also been hard at work preparing for the RCT in **Aim 3** scheduled for 2021. Starting in September, we added a 2-3hr weekly meeting to aid in planning for the RCT. To date, we have 1) carefully reviewed and prioritized all possible app refinements suggested by participants; 2) considered refinements to our planned RCT design based on data from past and current trials; and 3) are in the process of systematically reviewing our planned measurement battery and making changes to enhance efficiency and sensitivity. We have a review underway assessing for any stigmatizing language in the app and are developing some new content to add. Over the next 1-2 months, we will also be developing our protocol for the Coaching component we are planning to test in the RCT to enhance engagement.

We are also excited to share the progress of a graduate student in our lab, Carson Kautz-Turnbull, who is rigorously developing a measure of parental attributions called the Reasons for Children's Behavior (RCB) scale. The RCB is being developed to be easily administered online and will assess a critical construct we hypothesize is an important mechanism of change for FMF Connect. We plan to use this measure in our upcoming RCT. After developing an initial item pool, Ms. Kautz-Turnbull completed a first round of data collection (372 with usable data) and utilized factor analysis to refine the scale. This summer, she recruited new parents online (903 with usable data) and is currently utilizing item response theory (IRT) to identify the best performing items. Ms. Kautz-Turnbull is also in the process of applying for a Diversity F31 NRSA through NIAAA (due Dec 8) to develop and pilot the FMF Connect Teacher Companion Website.

2) Specific Objectives.

Aim 1: Using rigorous qualitative analysis of two rounds of beta-test data, determine: 1) what do users perceive as the strengths and weaknesses of the app prototype, and 2) what recommendations do they have for further refinements to improve usage and efficacy. Progress: Analyses are nearly complete and a manuscript is in preparation with submission goal of January 2021.

Aim 2: Test the feasibility of the FMF Connect app and the infrastructure required to recruit, screen, enroll, and collect quantitative data from participants. Progress: iOS users have completed the trial; Android is in progress. Results will inform refinements for the RCT in Aim 3.

3) Significant Results.

Aim 1. Systematic qualitative analysis of the two rounds of beta-testing is nearly complete. Evaluative and Values coding have revealed the following themes: Parents are driven to help their children be successful. They value information and support from others as a means to acquire the skills and resources to help their children. However, their time is limited and they need to accomplish this in a way that is efficient and doesn't add too much more stress to their lives. Understanding their child's disability is critical as FASD is confusing. Unfortunately, knowledgeable providers and resources are lacking. Given these constraints, parents are positive about the need for and utility of an app to help them achieve their informational and support goals. Positive evaluations were most salient for the components/content and the affective/social benefits of the app. These categories align well with their values relating to understanding their child and connecting with others, as well as their stated motivators for using the app. The most salient negatively evaluated aspects of the app center around efficiently navigating the app to get to what want when they want it (e.g., not liking step-by-step progression of content; number of videos; navigating forum). Almost all the identified barriers to using the app relate to these aspects. Participants offered a number of useful recommendations to improve navigation and enhance engagement with the app (e.g., table of contents, selected videos, new forum interface, open up content, notifications, coaching, etc). Many of these recommendations have been added or are in the development process.

Aim 2. A total of 172 participants have initiated the Screening & Consenting Module to date; 23 were ineligible, and 42 had incomplete data. iOS data collection metrics: A total of 63 iOS users had complete screening data and were eligible for the study. 52 completed baseline surveys (83% of eligible). Of these 52 participants, 41 installed the app (79%). Of the 41 who installed, 12 (29%) did not complete any Learning Modules; 14 (34%) completed all 4 Level 1 modules; 8 (34%) completed all Level 2 modules; and 6 (14%) completed all 12 modules. At the 3-month follow-up time point, 30 participants completed all surveys (58% of 52) and an additional 7 reported just on how their COVID-19 experiences impacted their participation in the study (combined 65% any response). Not unexpected, most parents reported the pandemic resulted in "a little more" (35.1%) to "a lot more" (51.4%) stress in parenting. More than half of parents indicated the pandemic decreased their ability to use the app (38.9% "a little less", 27.8% "a lot less"). A quarter reported their use was about the same as expected. On the User Version of the Mobile Application Rating Scale (uMARS; n=30), mean scores (out of max of 5) reflect a relatively high level of satisfaction with the FMF Connect app): Engagement = 3.90, Functionality = 4.23, Aesthetics = 4.25, Information = 4.57, Total App Quality = 4.24, and Perceived Impact 3.80.

4) Key Outcomes and Other Achievements.

Results across beta-test and feasibility trials indicate participants have relatively high satisfaction with the FMF Connect app. Theory- and data-driven directions for refinements have been identified and are in

progress for the upcoming RCT planned for 2021. Our team has productive over the last 6 months, including 3 presentations and 2 manuscripts submitted or in preparation.

Additional Questions

CIFASD4 Synergy. *Describe your project's interrelation with aims of the CIFASD consortium its other projects and the progress on those interactions.*

Our project is consistent with the mission of CIFASD to inform and develop effective interventions and treatment approaches for FASD. Within CIFASD-5, our project is both a recipient and referral source for participants. Since June 2020, our project has referred 67 participants from our FMF Connect trial to the DiG study. We anticipate 47 more in early 2021. We had hoped to collect saliva and 2D and 3D photos from participants in Dr. Petrenko's Tuning in to Kids clinical trial (benefitting projects led by PIs Foroud & Suttie), but unfortunately the COVID-19 pandemic has halted our in-person data collection. Our FASD Clinic is also in the process of getting IRB approval to collect data on the Morpheus Q app.

Pandemic - May 2021. *If nothing changes with regard to the pandemic, what do you hope your project can achieve by May 2021 (the end of the 4th year budget period for CIFASD4) and/or what Covid-19 impacts will remain obstacles?*

By May 2021, we anticipate beginning our RCT of the FMF Connect app. This is feasible given study participation was already planned to be done remotely. We are currently weighing the timeline of when specifically to initiate the trial. We have identified a sizeable number of possible refinements to the app, based on participant feedback in beta-testing and feasibility trials. We have prioritized this list and are working hard to implement as many as possible. We are considering how best to balance implementation of these refinements and the RCT timeline. If the decision is made to extend CIFASD-4, we would likely choose to implement more refinements and delay the RCT timeline. However, we would be able to complete the RCT prior to project end (with fewer refinements, but still a robust app).

While we will be able to complete all of our study aims by the end of the project, the COVID-19 pandemic will result in historical discontinuity, which will impact data interpretation and generalizability. The RCT design does benefit from a control group, which will lessen interpretation concerns, but it will be important to recognize findings may be different during this pandemic than they might have been otherwise.

Administrative Supplements. *For this reporting period (June 2020 to present), please list any Administrative Supplement associated with this award and a sentence or two about what it entails.* n/a

Publications/Abstracts June 2017 - present

Publications [Accepted & In Press]

Petrenko, C.L.M., Parr, J., Kautz, C., Tapparello, C., Olson, H.C. (2020). Development of the families moving forward connect mobile health intervention for fetal alcohol spectrum disorders: qualitative evaluation of design and functionalities. *JMIR mHealth and uHealth,* 8(4):e14721. PMCID: PMC7171567

Publications [In Preparation & Submitted]

Kautz-Turnbull, C., Petrenko, C.L.M., Handley, E.D., Coles, C.D., Kable, J.A., Wertelecki, W., Yevtushok, L., Zymak-Zakutnya, N., Chambers, C.D., & CIFASD. (submitted). Partner influence as a factor in maternal alcohol consumption and depressive symptoms, with subsequent effects on infant neurodevelopmental outcomes.

Petrenko, C.L.M., Parr, J., Kautz, C., Roth, A., Tapparello, C., Olson, H.C. (in preparation). Results from Two Rounds of Beta-Testing of the Families Moving Forward Connect App for Caregivers Raising Children with FASD.

Poster Abstracts and Presentations

Petrenko, C.L.M., Roth, A., Parr, J., Kautz-Turnbull, C., Tapparello, C., & Carmichael Olson, H. (2020). Families Moving Forward Connect: Development of a Mobile Health Intervention for Caregivers of Children with Fetal Alcohol Spectrum Disorders. Accepted for virtual poster presentation to be held at the upcoming Association of University Centers on Disabilities (AUCD) Annual Meeting: Achieving Equity: Leading the Way in the Next Decade, December 7-9, 2020.

Petrenko, C. L. M. (2020). Increasing Access to Care. Invited Plenary at the 2020 Proof Alliance Conference: ProofCon 2020: FASD in a New Era. Virtual conference, October 22-23, 2020.

Kautz, C. (2020, June). Reasons for Children's Behavior: The first phase of development for a new measure of parental attributions. Presentation to Department of Psychology, University of Rochester, Rochester, NY.

Kautz, C., Petrenko, C.L.M., Handley, E.D., Chambers, C., Coles, C., Kable, J.A., Wertelecki, W., Yevtushok, L., & Zymak-Zakutnya, N. (2020). Partner support as a predictor of maternal alcohol consumption and depressive symptoms during pregnancy, and subsequent effects on infant neurodevelopmental outcomes. Presentation at 43rd Annual Research Society on Alcoholism Scientific Meeting. Held virtually due to COVID-19.

Roth, A., Petrenko, C. L. M., Parr, J., Kautz, C., Tapparello, C., Olson, H. C. (2020). Results of two rounds of beta-testing of the Families Moving Forward Connect app for caregivers of children with FASD. Poster accepted for the 9th International Research Conference on Adolescents and Adults with FASD: Review, Respond, and Relate: Integrating Research, Policy, and Practice Around the World. Originally scheduled April 22-25 (postponed COVID-19), Vancouver, BC, Canada.

Petrenko, C. L. M., & Tapparello, C. (2019). Development of a mobile health intervention for caregivers of children with FASD; results from initial design and usability evaluations. *Alcoholism: Clinical and Experimental Research*, *43,* 324A. Oral presentation at the 42nd Research Society on Alcoholism, June 22-26, Minneapolis, MN.

Petrenko, C. L. M., Tapparello, C., & Parr, J. E. (2019). Families Moving Forward – Connect: Developing a mobile health intervention for families raising children with fetal alcohol spectrum disorders. 90-minute seminar presented at the 8th International Conference on FASD: Research: Results and Relevance. Integrating Research, Policy, and Promising Practice Around the World, March 6-9th, 2019. Vancouver BC.

Principal Investigator: Joanne Weinberg
Institution: University of British Columbia
CIFASD4 Project: Immune Dysregulation in FASD: Programming of Health and Neurobehavioral Outcomes
Grant Number: 5U01AA026101-04

Specific Aims

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

Our CIFASD Developmental Project (DP) is the first to identify links among maternal alcohol consumption, inflammation, and child outcomes; unique immune signatures in pregnant women were identified in association with both alcohol consumption and neurodevelopmental outcomes of their children. The proposed 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) Dr. 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, and long-term adaptive, functional, 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.

Accomplishments

1) Major Activities, June 2020-present.

Unfortunately, all recruiting and testing of adults with FASD (Aim 2) is still on hold due to the COVID-19 pandemic, as per Provincial and UBC guidelines. Active recruiting and testing of children by our collaborators (Aim 1) is also on hold or slowed considerably due to the pandemic. Nevertheless, we are making good progress in a number of areas to move our project forward:

a) In September, restricted lab work was allowed to resume and we connected with Jeff Wozniak, a collaborator on Aim 1 to inform him that we could resume our assay work. Plasma samples from his choline clinical trial - 67 samples from 34 unique subjects - were received in October and cytokine assays and pre-processing of the data have now been completed. We are now in communication with Jeff and his statistician to obtain additional sample information required for data analysis.

b) We have been developing and refining protocols for analysis of Th17 cytokines, a panel of inflammatory markers to be run on plasma samples from adults with FASD recruited in Vancouver, as well as by our collaborators, Claire Coles and Therese Grant, in Seattle and Atlanta, respectively (Aim 2). We intend to begin running these assays within the next month.

c) We have also been working on protocols to assay A1C, a measure of blood sugar regulation. We anticipate completing the A1C assays on plasma samples from all three sites in December/Januray.

d) Data analysis on the matched mother-child samples (Aim 1) from Tina Chambers' longitudinal Ukraine study is ongoing; Concept Proposal submitted: *Concept Proposal 90:* Raineki, C., Bodnar, T., Wertelecki, W., Yevtushok, L., Plotka, L., Granovska, I., Zymak-Zakutnya, N., Pashtepa, A., Wells, A., Honerkamp-Smith, G., Coles, C.D., Kable, J.A., Chambers, C.D., Weinberg, J., and the CIFASD. Differential associations between maternal and child Immune milieus in alcohol-dependent and alcohol-independent neurodevelopmental delay.

e) Pre-post COVID-19 study on the Vancouver cohort of adults with FASD.

Study ongoing. We are re-contacting all participants already tested (total n=72 [FAS, 18; FASD/ARND, 28; Unexposed, 26) to examine the impact of COVID-19 on mental health status, stress levels, and other related domains. Questionnaires being utilized were selected from the NIH COVID-19-related resources and focus on Covid-related stress and anxiety, the impact of COVID-19 on work and finances, and assessment of barriers to overcoming self-isolation/accessing health care (with a focus on questions geared to people with disabilities). We have also included open-ended questions from the "telling our stories in the age of COVID-19" questionnaire. As well, we will re-administer the Beck Depression, Beck Anxiety, Perceived Stress Scale, Penn State Worry questionnaires to assess the impacts of the pandemic on mental health status of our participants by comparing with outcomes collected prior to the pandemic.

2) Specific Objectives.

Our specific objectives during this period were to move forward with both Aims 1 and 2 as much as we possibly could. It has been extremely challenging to have to put recruitment and testing for our Adult Health study on hold due to Provincial and UBC COVID-19 restrictions. Understandably, all of our collaborators on whom we depend for blood samples are in the same situation and only able to move forward in a limited manner. However, in order to continue making progress, we have shifted our focus to include the above-described COVID-19-related study that supplements our Specific Aims and can be completed remotely (phone/Zoom), as well as to data analysis, assay development and completion, and manuscript writing.

3) Significant Results.

Further analyses of the data to date from our Adult Health Study (Vancouver data, total n=72) (Aim 2) indicate that overall, adults with FASD:

- are not significantly different from their control counterparts in either height or weight, nor do they show adverse changes in blood pressure or heart rate (**Fig 1**).

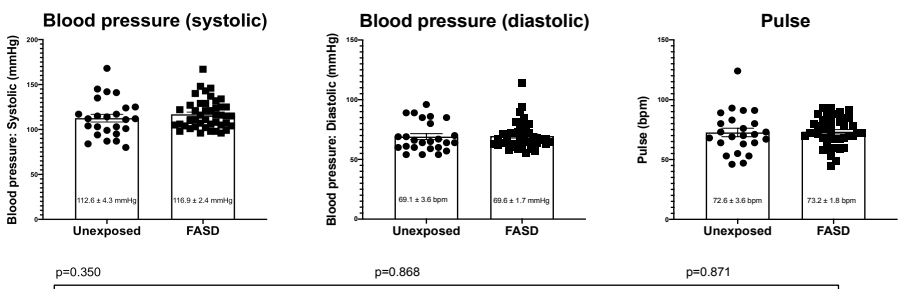

Fig 1. Blood pressure and heart rate in adults with FASD vs unexposed adults

- exhibit adverse changes in immune cell counts and increased preclinical symptoms of autoimmune disorders than their unexposed counterparts (**Fig 2**).
- exhibit and more physical and mental health problems than their unexposed counterparts (**Table 1**).

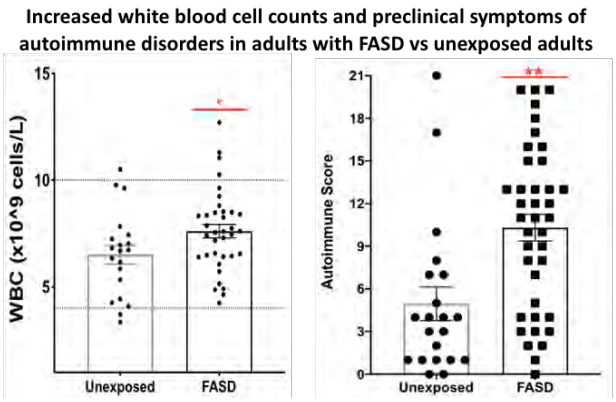

Fig 2. Adults with FASD show increased numbers of white blood cells and increased preclinical symptoms of autoimmune disorders compared to their unexposed counterparts.

Table 1. Selected physical and mental health problems in adults with FASD

Health survey question	Unexposed % = yes	FASD % = yes
# days physical health not good over the past month	7.4 (n=12)	16.1 (n=29)
Eye problems	31% (n=26)	61% (n=46)
Hearing problems	20% (n=25)	53% (n=45)
Teeth problems	50% (n=26)	80% (n=46)
Digestive problems	20% (n=25)	68% (n=46)
Childhood heart problems	0% (n=26)	11% (n=36)
Thyroid or parathyroid problems	0% (n=26)	14% (n=44)
Asthma	20% (n=26)	44% (n=45)
Epilepsy	4% (n=26)	24% (n=46)
ADHD	20% (n=25)	33% (n=42)
Depressive disorder	46% (n=26)	59% (n=44)
Anxiety disorder	50% (n=24)	58% (n=45)
Psychotic disorder or schizophrenia	0% (n=24)	4% (n=46)
>2 adverse childhood experiences	54% (n=26)	83% (n=46)

Additional analyses on data we have to date from adults with FASD are currently underway. As well, as noted above, analyses and manuscript writing are moving forward on studies for Aim 1.

4) Key Outcomes and Other Achievements.

a) Publication of Chapter reporting the results of the Health Survey conducted by the Adult Leadership Committee: Himmelreich, M., Lutke, C.J., Hargrove, E.T. Chapter 12: The Lay of the Land. Fetal Alcohol Spectrum Disorder (FASD) as a Whole-Body Diagnosis. In: Begun, A.L. & Murray, M.M. (eds). The Routledge Handbook of Social Work and Addictive Behaviors. Routledge, NY, 2020.

b) In addition to publications and a manuscript in progress, key outcomes include excellent progress toward our goals in Aims 1 and 2, as well in dissemination of our results in symposia, talks and posters at numerous local, national and international meetings (see below).

Additional Questions

CIFASD4 Synergy. *Describe your project's interrelation with aims of the CIFASD consortium its other projects and the progress on those interactions.*

1) Collaboration with *Tina Chambers and team* on her U01. Blood samples obtained for cytokine assays on pregnant women, children, and matched mother-child pairs from her longitudinal birth cohort study in Ukraine. Two publications and one manuscript in preparation.
2) Collaboration with *Ken Jones and Tina Chambers* on a health-related study of children, ~5-17 years, recruited from the San Diego FASD Research Subject Pool at Rady Children's Hospital-San Diego. Will have access to banked maternal mid-gestation plasma samples and child newborn blood spots. Unexposed (control group) children will be recruited by Tina through other ongoing studies. Data collection ongoing.
3) Dysmorphology Core. Three member of the Weinberg lab spent several days in San Diego to train with *Ken Jones* on facial dysmorphology. Our team has completed facial dysmorphology measures for a number of subjects in our Adult Health Study via telemedicine consultation with Ken.
4) Collaboration with *Jeff Wozniak* on his choline clinical trial. Plasma samples from children with FASD receiving either choline or placebo were received in our lab in October. Cytokine assays and preprocessing of data now completed. Data analysis ongoing.

5) Collaboration with *Claire Coles* (Emory University, PI) and *Therese Grant* (University of Washington, Co-I) on the Adult Health Study. In parallel, we are each recruiting cohorts of adults with FASD and appropriate unexposed controls. Assessment of immune function/inflammation, physical and mental health, cognitive and adaptive function. Cytokine assays completed on the first set of 40 samples from all 3 sites. Protocols for analysis of a Th17 panel and A1C in development. We aim to run assays in December/January. Comparison of mental and physical health outcomes among the 3 sites underway.
6) Weinberg is providing 2D facial pictures of adults with FASD and controls to *Mike Suttie* for analysis under his U01.
7) Interaction with *Tatiana Foroud* and *Leah Weatherill*. We are collecting saliva samples on all of our participants for their genetic analyses. Out of a possible 46, we have collected/sent 44 (19 FAS, 25 FASD) (2 participants unable to complete the testing day).
8) Aliquots of plasma from blood samples taken from infants in *Tina's* new Ukraine birth cohort will be analyzed by Weinberg (cytokines), *Miranda* (miRNA), and *Hashimoto-Torii* (epigenetic marks). Data will assess whether the use of multiple biomarkers can provide more sensitive indicators of risk/resilience than any single biomarker alone.

Pandemic - May 2021. *If nothing changes with regard to the pandemic, what do you hope your project can achieve by May 2021 (the end of the 4th year budget period for CIFASD4) and/or what COVID-19 impacts will remain obstacles?*

<u>If nothing changes with the pandemic:</u>

Even if nothing changes with regard to the pandemic, we will still be able to make strong progress on a number of fronts:

Analysis of cytokine levels in mothers and children and child outcome data on matched mother-child pairs from Tina's Ukraine longitudinal cohort study is ongoing. We aim to submit a manuscript on these data in the late spring.

Assays of Th17 cytokine levels and A1C levels on plasma of adults with FASD/unexposed controls from the 3 sites will be completed in December/January and data analysis will follow. Depending on the data obtained, we will work with our collaborators, Claire and Therese, to determine how we can move forward.

We will complete our pre-/post-COVID-19 study on adults with FASD in the next few months and aim to submit a manuscript for publication during the summer. We will also work with Claire and Therese who are undertaking COVID-related studies on their cohorts of adults with FASD to determine if we can develop a publication based on data from all 3 sites.

Whenever the Provincial government and UBC allow us to resume recruiting and testing we are poised to do so, and aim to move the study forward as rapidly as possible to reach our recruiting targets.

<u>COVID-19 impacts that will remain obstacles:</u>

If the Province of BC and UBC will not allow resumption of recruitment and testing over the next few months, we will fall short of our target for recruiting and testing participants for our Adult Health Study (Aim 2). As well, we are currently short of our targets for the studies in Aim 1, which depend on blood sample and data collection by our collaborators, who have also been unable to resume and/or delayed in resuming in-person studies.

With this possibility in mind, we have reached out to colleagues elsewhere who are currently able to do in-person research, and who may be able to help fill the gaps for us:

Dr. Natasha Reid is a Clinical Psychologist and Research Fellow, Child Health Research Centre, Faculty of Medicine, University of Queensland (UQ). Dr. Reid is affiliated with the research team of Dr. Karen Moritz, who is Director of this Centre. Dr. Reid's research interests include prevention of prenatal alcohol exposure, interventions for children with FASD, and assessment and diagnosis of FASD. Currently, Dr. Reid is working with a diagnostic clinic at UQ that provides assessment services for the treatment and management of children with FASD. In partnership with Pathology Queensland, Dr. Reid is also involved in research to develop a diagnostic tool that could examine markers in the blood to determine if a child has been exposed to alcohol in pregnancy. Thus, under her research protocol, blood samples are being collected from children

who are assessed at this clinic. In collaboration with Dr. Reid, we could obtain blood spots as well as demographic and health information (including body composition measurements), and cognitive, language, and behavioral assessments from children with FASD as well as matched controls seen at the clinic. Importantly, we already have a good working relationship with Dr. Reid through interactions over many years at RSA and other meetings, where our lab members have had extensive connections, as well as interactions in the planning of a symposium organized by Dr. Tammy Bodnar for the 2020 RSA meetings.

While there is ongoing collection of blood samples and health data from the San Diego FASD Research Subject Pool (Rady Children's Hospital - Ken Jones and Tina Chambers, Aim 1), additional blood samples and outcome data from children being assessed in Dr. Reid's research could increase our participant numbers and serve as a comparison group to provide further insight into environmental/demographic factors that can influence child outcomes.

Dr. Kaitlyn McLachlan is an Assistant Professor in the CPA-accredited Clinical Psychology program at the University of Guelph and a Research Lead for the Canada FASD Research Network (CanFASD). Her current program of research centers around FASD and neurodevelopmental disability across the lifespan, with a focus on the criminal justice system. Dr. McLachlan's research interests include understanding neurobiological and environmental risk markers associated with adverse outcomes in FASD, the importance of establishing valid and reliable screening and diagnostic approaches, and better understanding risk and protective factors associated with criminal justice system trajectories. Importantly, we already have an excellent working relationship with Dr. McLachlan through a previous successful collaboration to evaluate HPA function in children with FASD compared to that in typically developing controls through assessment of diurnal cortisol activity, as well as the associations among specific early life adversities, adverse outcomes, protective factors, and diurnal cortisol (McLachlan et al., Alcohol 53:9-18, 2016). Working in collaboration with Dr. McLaclan, we can reach out to cohorts of adults with FASD and appropriate unexposed adults to recruit them to our Adult Health Study. Testing and blood sample collection could be done through her laboratory at Guelph. With this collaboration, adults recruited in Ontario could help us reach our target numbers and could also serve as a comparison group to provide further insight into environmental / demographic factors influencing adult health and functional outcomes.

While our goal is to complete all of our recruiting, testing, and sample analysis for Aims 1 and 2 as proposed in our Specific Aims, we felt it was critical to develop additional options for recruiting and testing given the current and possibly long-lasting COVID-19--related restrictions. We believe that these additional collaborations may be critical in helping us reach our target numbers if COVID-related restrictions hamper our ability to move the research forward as proposed. Though we will have to account for the differences in populations between our current cohorts and these new cohorts, we view these additional collaborations as opportunities to gain further insight into risk/resilience variables in our populations.

Administrative Supplements. No supplements.

Publications/Abstracts June 2017 - present

Publications [Accepted & In Press]

Bodnar, T.S., Raineki, C., Wertelecki, W., Yevtushok, L., Plotka, L., Granovska, I., Zymak-Zakutnya, N., Pashtepa, A., Wells, A., Honerkamp-Smith, G., Coles, C.D., Kable, J.A., Chambers, C.D., Weinberg, J., the CIFASD (2020). Immune network dysregulation associated with child neurodevelopmental delay: Modulatory role of prenatal alcohol exposure. *Journal of Neuroinflammation* 17:39. doi: 10.1186/s12974-020-1717-8. PMCID: PMC6988366

Bodnar, T.S., Raineki, C., Wertelecki, W., Yevtushok, L., Plotka, L., Zymak-Zakutnya, N., Honerkamp-Smith, G., Wells, A., Rolland, M., Woodward, T.S., Coles, C.D., Kable, J.A., Chambers, C.D., Weinberg, J., CIFASD. (2018). Altered maternal immune networks are associated with adverse child neurodevelopment: Impact of alcohol consumption during pregnancy. *Brain Behav Immun* 73:205-215. PMCID: PMC6344127

Publications [In Preparation & Submitted]

Concept Proposal 90: Raineki, C., Bodnar, T., Wertelecki, W., Yevtushok, L., Plotka, L., Granovska, I., Zymak-Zakutnya, N., Pashtepa, A., Wells, A., Honerkamp-Smith, G., Coles, C.D., Kable, J.A., Chambers,

C.D., Weinberg, J., and the CIFASD. Differential associations between maternal and child Immune milieus in alcohol-dependent and alcohol-independent neurodevelopmental delay (in preparation).

Poster Abstracts and Presentations

Posters:

Bodnar, T.S., Raineki, C., Chao, A., Loock, C., Oberlander, T., Weinberg, J., the CIFASD (2020). Evidence for long-lasting changes in health outcomes and immune function in adults with Fetal Alcohol Spectrum Disorder. 43rd Annual Research Society on Alcoholism Meeting, New Orleans, LA, June 20-24. CANCELLED

Bandoli, G., Bodnar, T., Raineki, C., Wertelecki, W., Yevtushok, L., Zymak-Zakutnya, N., Weinberg, J., Chambers, C. (2020). The role of iron supplements in modifying the association between maternal alcohol consumption, IL-6 expression and child birth outcomes. Research Society on Alcoholism Meeting, New Orleans, LA, June 20-24. CANCELLED

Bodnar, T.S., Raineki, C., Jones, M.J., Lewinn, K., Davis, R.L., Kobor, M.S., Smith, A.K., Tylavsky, F.A., Bush, N.R., Weinberg, J. (2019). Associations between socioeconomic status and immune activity during pregnancy: Implications for child development. Developmental Origins of Health and Disease World Congress, Melbourne, Australia, October 20-23,.

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

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

Raineki, C., Bodnar, T.S., Wertelecki, W., Yevtushok, L., Plotka, L., Zymak-Zakutnya, N., Wells, A., Honerkamp-Smith, G., Coles, C.D., Kable, J.A., Chambers, C.D., J. Weinberg, the CIFASD (2018). Alcohol consumption during pregnancy is associated with altered maternal and child immune function. 2018 Alcohol & the Nervous System: Gordon Research Conference. Galveston, TX, March 4-9.

Symposia

Bodnar, T.S., Boschen, K.E., Manke, A., Petrenko, C., Przybysz, K.R., Raineki, C., Reid, N. (2020). Beyond prenatal alcohol exposure: Genes, environment, and diagnostic schemes as factors mediating outcomes. 43rd Annual Research Society on Alcoholism Meeting, New Orleans, LA, June 20-24. [Bodnar, T.S., Organizer]. CANCELLED

Raineki, C., Bodnar, T.S., Holman, P.J., Weinberg J. (2020). From animal models to humans: Role of early-life on emotional regulation and immune function following prenatal alcohol exposure. In: Beyond prenatal alcohol exposure: Genes, environment, and diagnostic schemes as factors mediating outcomes. 43rd Annual Research Society on Alcoholism Meeting, New Orleans, LA, June 20-24. CANCELLED

Weinberg, J., Coles, C., Grant, T.M., Bodnar, T., Raineki, C., Holman, P., Ellis, L., Yu, W., Lynch, M.E., Kneeland, G., Radin, S., Smith-Stewart, T., Kelly, K. (2020). Health outcomes in adults with FASD: Preliminary evidence across three study sites (2020). In: Health outcomes of adults with fetal alcohol spectrum disorders: A fetal basis to adult disease. 43rd Annual Research Society on Alcoholism Meeting, New Orleans, LA, June 20-24. [Miranda, R.C., Organizer] CANCELLED

Bodnar, T.S., Raineki, C., Wertelecki, W., Yevtushok, L., Plotka, L., Zymak-Zakutnya, N., Wells, A., Honerkamp-Smith, G., Coles, C.D., Kable, J.A., Chambers, C.D., Weinberg, J. and the CIFASD (2019). Prenatal alcohol exposure disrupts the immune milieu: Impacts over the life course. In: (Weinberg, Organizer and Chair): Neuroimmune dysfunction and health outcomes following prenatal alcohol exposure: Complementary cross-center perspectives. 42nd Annual Scientific Meeting of the Research Society on Alcoholism. Minneapolis, MN, June 22-26.

Raineki, C, Holman, PJ, Bodnar, TS, Weinberg, J. (2019). The impact of early-life adversity on emotional regulation and immune system function is mediated by adverse prenatal environment. In: Early life stress-based models of psychiatric disorders: Different paradigms and converging outcomes. 48th Annual Meeting of the European Brain and Behavior Society, Prague, Czech Republic, September 21-24.

Raineki, C., Bodnar, T.S., Wertelecki, W., Yevtushok, L., Plotka, L., Zymak-Zakutnya, N., Wells, A., Honerkamp-Smith, G., Coles, C.D., Kable, J.A., Chambers, C.D., J. Weinberg, the CIFASD (2018). Alcohol consumption during pregnancy is associated with altered maternal and child immune function. 2018 Alcohol & the Nervous System: Gordon Research Conference. Galveston, TX, March 4-9.

Oral Presentations

Raineki, C. (2020). Uncovering biological mechanisms for how the early environment shapes developmental trajectories: A translational approach. Department of Psychology, West Virginia University, Morgantown, WV, February 5,.

Weinberg, J. (2020). Prenatal alcohol exposure, fetal programming and later life vulnerabilities: From basic science to clinical research. Women's Health Seminar Series. February 24.

Weinberg, J. (2020). Adult Health Study. At: Honoring the Downtown Eastside Community: A gathering to address FASD in the circle of our lives. Vancouver, BC, February 27,.

Raineki, C. (2020). Evaluating the interactive effects of prenatal alcohol exposure and early-life adversity on emotional regulation. Department of Cellular & Physiological Sciences Research Retreat, Vancouver, BC, February 28.

Raineki, C., Holman, P.J., Bodnar, T.S., Weinberg, J. (2019). Modulatory role of postnatal environment on the effects of PAE: Risk vs. resilience. 8th International Conference on Fetal Alcohol Spectrum Disorder, Vancouver, BC, Canada, March 6-9.

Bodnar, T.S. (2019). Impact of prenatal alcohol exposure on immune function throughout the life course. 8th International Conference on Fetal Alcohol Spectrum Disorder, Vancouver, BC, Canada, March 6-9.

Bodnar, T.S., Holman, P.J., Raineki, C., Weinberg, J. (2019). Using a translational approach to evaluate the effects of prenatal alcohol exposure: Risk vs. resilience. Neuroscience (Neuropizza) Seminar Series, University of British Columbia, Vancouver, BC, December 4.

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

Bodnar, T.S. Raineki, C., & Bandoli, G. (2018). Maternal alcohol consumption and maternal depression: Emerging role of immune activation on modulating offspring outcome. 7th International Conference on FASD: Research, Results and Relevance, Vancouver, BC, March 1-4.

Weinberg, J. (2018). Developmental origins of health and disease - DOHaD: Prenatal alcohol effects on brain and behavior. Department of Cellular and Physiological Sciences Research Retreat, January 20.

Coles, C.D., Grant, T., & Weinberg, J. (2018). Mapping the Undiscovered Country: Health and Mental Health in Adults with FASD. 7th International Conference on FASD: Research, Results and Relevance, Vancouver, BC, March 1-4.

Weinberg, J. (2017). Effects of prenatal alcohol exposure on health outcomes across the lifespan: From animal models to the clinic. FASD Collaborative Roundtable. Douglas College, New Westminster, BC, Canada, November 25, 2017.

Principal Investigator: Tatiana Foroud, Co-I: Leah Wetherill
Institution: Indiana University
CIFASD4 Project: Dissecting the Genetic Contributions to Fetal Alcohol Spectrum Disorders (DiG-FASD)
Grant Number: 5U01AA026103-04

Specific Aims

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

Accomplishments

Aim 1: Develop a web portal with a novel, online consenting process to create a large CIFASD cohort for new studies.

Since the web portal went live, 564 individuals have enrolled, primarily from the United States and Canada. The majority of participants report themselves as Caucasian (65%); but our participants are diverse and also report Native American or First Nation (7%), African (8%) and other races (20%). The sample is 51% female. The study includes a consent for adult participants or parents of minors as well as an assent. As a result, participants include individuals ages 1-77 years, with 64% under 18 years (minor). Following consent, online forms gather information on alcohol exposure. 42.2% of participants report minimal exposure and 17.6% report heavy prenatal exposure. Similar to in person recruitment, 40% of participants do not have detailed information regarding prenatal alcohol exposure. Since June 1, 2020, 43 participants have enrolled through the online web portal.

Once individuals complete the consenting process and the online forms, they are invited to upload a 2D facial photograph and provide a saliva sample. We have 2D photos from 326 individuals and saliva from 240 individuals. Since June 1, 2020, 35 individuals have uploaded a photograph. We obtained saliva from an additional 8 individuals. Of note, saliva collection was halted at the start of the pandemic due to concerns about potential viral transmission. We have since reinstituted saliva collection.

During the COVID pandemic, we have made progress in several areas which will have significant impact over the remainder of the grant period. In an effort to increase the completion of study activities, we adjusted

the compensation so that rather than receiving an equal amount for each component ($10/activity, $50 total), we are now offering $30 for saliva, since DNA is fundamental to our study. Based on feedback obtained from the 2019 Vancouver International Conference on Fetal Alcohol Spectrum Disorders and the in-person CIFASD meeting in Fall 2019, we have also simplified our participant facing materials. These materials are pending IRB submission, and include:

- Consent: created a 3-part video for consenting (2-3 minutes each)
- Study Flyers: simplified language and updated visual appeal
- Face photo and Saliva instructions: simplified materials
- Online Forms: shortened and simplified

We have many collaborations with others in CIFASD. We continue to receive saliva samples from other CIFASD4 projects (Weinberg, Wozniak, Coles, Petrenko). The University of Washington group (Grant) recently began to collect saliva. Since June 1, 2020, we have also worked closely with the Mattson project to implement the online Brief Assessment of Individual Neurobehavior (BRAIN) assessment. We have worked with Dr. Mattson to finalize how our research participants will consent and complete the BRIAN assessment. When ready, we will offer our participants to complete this assessment, which will provide neurobehavior data that can be used for analyses in the Mattson project and our own. Given our common recruitment of adults in CIFASD, we have also worked with the CIFASD collaborators to include a subset of questions from their health outcomes assessment. These data will be pushed to the central repository and will increase sample sizes for variables collected by the CIFASD adult components.

Aim 2: Perform whole exome sequencing (WES) 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.

During the COVID-19 period, we updated our genome-wide association study (GWAS) data on 545 exposed and unexposed individuals from CIFASD phases 2-3 to complement the WES, which is focused on rare variants. Considerable effort was spent to reformat files and re-impute the data using the most up-to-date technology. We now have more than 4 million variants available for analyses. We will mimic the WES analyses comparing those with FAS to those without FAS in individuals with prenatal alcohol exposure and perform analyses utilizing spatial working memory measures in all individuals with available data to examine the gene*alcohol interaction in the genes identified above.

We previously analyzed WES data from 273 participants recruited as part of CIFASD 2 and 3. These results identified three genes: *CRIPAK* ($p=4.5x10^{-7}$) involved in upregulation of estrogen, *HTT* ($p=6.9x10^{-5}$) which encodes a hub molecule in rodents linking pathways affected by prenatal alcohol exposure regardless of timing (Kleiber et al, 2013), and *KIF2A* ($p=2.9x10^{-4}$), which directly interacts with ciliary proteins to regulate mTOR. Since June 1, 2020, we have generated WES data from an additional 207 CIFASD 4 participants (38% recruited from other CIFASD projects, and 62% from DiG-FASD). We combined these samples with the previous dataset (total N=480) using the same GATK based pipeline, and re-generated variant calls to improve the quality. We implemented the same gene-level burden test to compare FAS (vs No FAS). To maintain consistency with previous diagnoses, this initial screen only utilized the subset of individuals for which a CIFASD consortium diagnosis was available and only included individuals of European (N=70) or African (N=67) ancestry. Results which included the additional 13 FAS and 13 no FAS individuals did not support the original findings (CRIPAK p=0.70, HTT p=0.81, KIF2A $p=7.3x10^{-3}$). Unfortunately, these changed results illustrate the challenges of working with relatively small sample sizes.

We have also performed analyses in this same expanded sample to explore hypotheses from our CIFASD collaborators.

- We extracted results for genes of interest nominated by the animal models component (Eberhart and Parnell): *EFCAB7* (p=0.12*), UVSSA* (p=0.79), and *TTC19* (p=0.19*)*.
- We report our significant results to this component and as a result,
    - they are investigating Htt knockouts in mice and
    - exposed Kif2a mutant zebrafish.

- Dr. Torrii found that APOE is down-regulated in exposed mice, and that APOE expression is correlated with spatial motor learning task results in mice. WES results for risk of *APOE* variants being associated with FAS risk were not significant (meta p=0.67).

Pending analyses will use the entire sample of subjects with WES and will include the broad range of prenatal alcohol exposure. The focus of these analyses will be on the variables from the 2D photographs collected as part of this online project as well as several of the other CIFASD projects, and include data from 3D images converted to 2D. These analyses are underway as part of the Suttie project. Dr. Suttie will provide a dysmorphology score for the entire face, as well as scores for individual regions. We are ready to perform analyses once these data are available.

A key discussion point for the 'in person' meeting will be our plans for WES in another 132 samples. Generating these data would provide WES for 612 individuals.

Aim 3: Maintain a central informatics resource to manage sharing of data within CIFASD and with the broader research community.

The Central Repository is in place and functioning.

- All CIFASD investigators have uploaded a data dictionary
- All CIFASD investigators submit data quarterly, as applicable
    - Adult Study (Coles/Grant) N = 103
    - Neurobehavior (Mattson/Wozniak) N=110
    - Neuroimaging (Wozniak) N=96
    - eTree (Mattson) N=315
    - Immune (Weinberg) N=29
- These data are available to all other CIFASD investigators to be shared across components
- A folder was made to upload documents for social media, slides, etc to share with NOFAS, NIAAA

Since June 1, 2020, we have finalized the process by which external investigators request Archived CIFASD data. Process for requesting data from external investigators is finalized with a standard operating procedure (SOP). Three requests have been submitted since June:

- Sarah Oh (South Korea) – investigator supplied insufficient details to use the data requested. The information was requested and this application is pending.
- Paola Haeger (Chile) – investigator requested a letter of support to study stress profiles in children with prenatal alcohol exposure. Data will be provided pending grant funding.
- Laura Dyer (University of Portland) – investigator requested heart rate data from Ukraine to examine effects of prenatal alcohol exposure. This request was approved and data are being provided.

Key Outcomes and Other Achievements.

- Significantly improving our tools and efforts for outreach. Anticipate that this will pair well with our new expanded effort to increase participation. A new study coordinator has been instrumental in these efforts.
- Analyses including additional WES has not supported our previous findings. We are eager to analyze the larger sample using the dysmorphology phenotypes.
- There is greater participation in data transfer to the central repository. Central repository is able to support review of data requests. Next step is broader advertisement of CIFASD resources.

Additional Questions

CIFASD4 Synergy. *Describe your project's interrelation with aims of the CIFASD consortium its other projects and the progress on those interactions.*

A. *CIFASD projects collect saliva for the DiG-FASD online project*.

- Four other CIFASD projects (*Wozniak, Coles, Weinberg, Grant*) provide saliva samples from individuals with prenatal alcohol exposure. This is vital to increase the sample size of the genetic analysis.

- As the DiG-FASD project incorporates subsets of these projects into the web portal protocol, it also increases the sample size of the adult CIFASD projects focused on health-related outcomes (*Coles, Weinberg*). This will allow us to explore genetic differences in these outcomes.
- We will work closely with Dr. Chambers to replicate results from whole genome sequencing in the Ukrainian sample (*Chambers*).

B. *We continue to share 2D facial photos with all CIFASD components.*

- All 2D facial photos assessed through the web portal are available as part of the secure Central Repository to other CIFASD investigators.
- We are working with Mike Suttie (*Facial Imaging*) to develop a facial risk score from the photos. We will analyze these scores in our WES and GWAS samples. The scores will be part of the quarterly upload of data to the Central Repository.

C. *We are implementing an online neurocognitive assessment (Mattson).*

- As we incorporate the BRAIN into the DiG-FASD web portal, these scores will be analyzed in the WES and GWAS data. They will also be included in the quarterly upload of data to the Central Repository.

D. *Outreach/dissemination*:

- Results from the additional NOFAS compensation from the previous year were presented as a poster at RSA, 2020.
- Tom Donaldson and Kathy Mitchell continue to promote our DiG-FASD study in their talks and in meetings by showing the promotion slide.
- NOFAS has created several images to help advertise the DiG-FASD study on Facebook and other social media outlets.

E. *Animal model components*. We are working closely with the animal model components (Eberhart, Parnell, Torrii) to run analyses that approximate the phenotypes utilized in their models.

- Genes identified from the animal model components are tested for association in WES and GWAS data in humans.
- Variants/genes detected from WES analyses are shared with the animal model components to be assessed and confirmed.

F. *We are working with Developmental Projects.*

- We are communicating with Dr. Susan Smith to assist in her analyses of GWAS data.

Pandemic - May 2021. *If nothing changes with regard to the pandemic, what do you hope your project can achieve by May 2021 (the end of the 4th year budget period for CIFASD4) and/or what COVID-19 impacts will remain obstacles?*

A significant impact for us from COVID-19 were delays in recruiting a new study coordinator when our current study coordinator moved to another position. We had to obtain approval to post a position, which was a relatively slow process. We now have a very capable person in place. We were delayed in several new initiatives that were underway and we largely halted recruitment and the collection of saliva samples for several months. We believe that we are now in an improved position with regard to study recruitment.

We are concerned about the challenges of recruiting and do not believe we can achieve our initially proposed recruit goals. This is not solely due to COVID-19, but is impacted by it.

Administrative Supplements. *For this reporting period (June 2020 to present), please list any Administrative Supplement associated with this award and a sentence or two about what it entails.* n/a

Publications/Abstracts June 2017 - present

Publications [Accepted & In Press]

Sarkar DK, Gangisetty O, Wozniak JR, Eckerle JK, Georgieff MK, Foroud TM, Wetherill L, Wertelecki W, Chambers CD, Riley E, Zymak-Zakutnya N, Yevtushok L. Persistent changes in stress-regulatory genes in pregnant women or children exposed prenatally to alcohol. *Alcohol Clin Exp Res*. 2019. Sep;43(9):1887-1897. doi: 10.1111/acer.14148. Epub 2019 Aug 6. PMID: 31329297; PMCID: PMC6722014.

Suttie M, Wozniak JR, Parnell SE, Wetherill L, Mattson SN, Sowell ER, Kan E, Riley EP, Jones KL, Coles C, Foroud T, Hammond P; CIFASD. Combined face-brain morphology and associated neurocognitive correlates in fetal alcohol spectrum disorders. *Alcohol Clin Exp Res*. 2018 Sep;42(9):1769-1782. doi: 10.1111/acer.13820. Epub 2018 Jul 20. PubMed PMID: 29935097; PMCID: PMC6120799.

Dou X, Menkari C, Mitsuyama R, Foroud T, Wetherill L, Hammond P, Suttie M, Chen X, Chen SY, Charness ME; 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*. 2018 Mar;32(3):1364-1374. doi: 10.1096/fj.201700970. Epub 2018 Jan 3. PubMed PMID: 29109170; PMCID: PMC5892731.

Wetherill L, Foroud T, Goodlett C. Meta-analyses of externalizing disorders: Genetics or prenatal alcohol exposure? *Alcohol Clin Exp Res*. 2018 Jan;42(1):162-172. doi: 10.1111/acer.13535. Epub 2017 Nov 27. PMID: 29063614; PMCID: PMC5750073.

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. PMID: 28608920; PMCID: PMC5563255.

Poster Abstracts and Presentations

Wetherill L, Rowe E, Donaldson T, Mitchell K, Schulze J, EP Riley, Foroud T, NOFAS, CIFASD. Novel Online Recruitment for FASD-Related Studies and the Effect of Compensation. Research Society on Alcoholism 2020, Poster Presentation.

Wetherill L, Rowe E, Schulze J, CIFASD, Foroud T, Genetics and FASD: Understanding why FASD outcomes are different. 8th International Research Conference on Fetal Alcohol Spectrum Disorders, Vancouver, 2020.

Wetherill L, Goodlett C, Mattson SN, COGA, CIFASD. Prenatal alcohol exposure increases risk for ADHD after accounting for genetic liability. American Society of Human Genetics Meeting, Houston, TX, Oct 15-19, 2019.

Wetherill L, Nudelman K, Parnell SE, Coles C, Jones K, Kable J, Sowell E, Wozniak J, Riley EP, Mattson SN, Foroud T, CIFASD. Huntingtin coding variants and spatial working memory measures in prenatally exposed individuals. FASt presentation, FASD Study Group (Satellite Meeting), 42nd Annual Research Society on Alcoholism Scientific Meeting, Minneapolis, Jun 22-26, 2019.

Wetherill L. Tech Talks: 3D Imaging in Medical Research. IUPUI Technology in Medicine Student Interest Group, Indianapolis, April 16, 2019.

Foroud T, Rowe E, Wetherill L, Schwantes-An T. The genetics of FASD: accelerating research advances. 8th International Conference on Fetal Alcohol Spectrum Disorder, Vancouver, March 6-9, 2019.

Foroud T. Registry Updates: Advancing Research through Participation. 8th International Conference on Fetal Alcohol Spectrum Disorder, Vancouver, March 6-9, 2019.

Wetherill L, Mattson SN, Foroud T, Goodlett C. Effect of Prenatal Alcohol Exposure and Parental Alcohol Dependence on Rates of Externalizing Disorders in COGA and CIFASD Samples. Research Society on Alcoholism 2018, Poster Presentation.

Website: https://digfasd.org/

Principal Investigator: Claire D. Coles; Co-I: Therese Grant
Institutions: Emory University School of Medicine; University of Washington
CIFASD4 Project: Fetal Alcohol Spectrum Disorders in Adults: Health and Neurobehavior
Grant Number: 5U01AA026108-04

Specific Aims

1. Establish a Registry of individuals with prenatal alcohol exposure (PAE) who are willing to participate in future research.
2. Tier 1: With 500 individuals from Seattle and Atlanta cohorts older than 30 years of age with prenatal exposure or FASD diagnosis as well as unexposed contrast groups carry out a demographic and adult health survey.
3. In a subsample of adults selected from 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.

4. 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.

Accomplishments

1) Major Activities. In 2020, within the parameters allowed by the COVID-19 pandemic, our major focus has been on recruitment and data collection. In addition, we continue to work with collaborators at other CIFASD projects to accurately collect and share data.

a. **Modification of Protocols to accommodate changes necessary due to COVID-19 pandemic.** Considerable effort was devoted this year to modifying the protocols to accommodate to these unexpected conditions. The Universities both stopped activities in March. Once we were allowed in the laboratories again, we adapted materials and consent forms to allow remote collection of questionnaire data. Eventually, we were able to initiate in-person data collection for Tier 2 and the COVID and Diversity supplements (October 1). However, due to the safety protocols necessitated by the pandemic and the requirements of the universities, the rate of data collection is substantial reduced as we are able to see only a single participant at a time and have to do so when the site is not engaged in other activities. Continuing data collection at this rate is dependent on the status of the pandemic in each location.

b. **Cross-Site Collaboration**. We continue to coordinate activities between Atlanta and Seattle including the addition of the COVID supplement and associated materials and we continue our collaboration

with Dr. Weinberg's lab to allow for eventual group analyses of data. This collaboration is maintained through frequent contact as well as, currently, quarterly inter-site conference calls among the 3 sites.

c. **Data Collection for Tier 1 and Tier 2.** Despite the pandemic-related shutdowns required by the University of Washington and Emory University, data collection continued remotely for both Tier 1 and Tier 2. Direct in-person assessment for Tier 2 was reinitiated in October 2020. There have been no problems in implementing remote protocols. As of November 2020, we have completed 269 Tier 1 and 164 Tier 2 evaluations; For Tier 1, data collection is currently at 65% of FY21 goal; For Tier 2, it is at 85% of goal.

d. **Telemedicine for dysmorphology examinations for FASD**. Dysmorphology evaluation are being done in Seattle by project staff. We have not been able to collect this information as yet in at Atlanta. Training by Dr. Jones was completed by our project staff (Gaby Ritfeld, MD) in February 2020 to allow telemedicine assessment of dysmorphology but we have not implemented this aspect of the study due to the COVID pandemic restrictions.

e. **Collaboration with Associated Studies.** In the current grant year, we continued to collaborate with two other investigators who have initiated "associated" studies that rely on this CIFASD longitudinal study.

- In Atlanta, a R21 with Julie Kable, PhD as the Principle Investigator, collected data on micro vascularization and cardiac health as well as frontal lobe functioning in adults participating in Tier 2 until the study period concluded in Fall, 2020. Co-Investigators included Dr. Coles and Puja K. Mehta, MD, Director of Women's Translation Cardiovascular Research of the Department of Cardiology, as Co-Investigators.

- In Seattle, Drs. Grant and Radin are collaborating with Eileen Moore, PhD (PI, San Diego State University) on her R01 project "Brain Maturation in Adults with FASD." to recruit 90 diagnosed individuals and controls who 1) had previous structural MRI scans done in our earlier UW studies; and 2) are now completing neurobehavioral testing as part of Phase IV CIFASD. This study was paused by the University due to the pandemic and was restarted in November 2020. As of this point, 33 scans have been done including 9 individuals with FAE, 14, with FAS, and 10 controls.

2) Specific Objectives. These do not differ from the Specific Aims.

a. Establish a Registry and collect Health Survey (Tier 1): This has been done at both sites and data combined in REDCap. As of this date, 269 individuals have completed this level.

b. Tier 2: In–Depth Testing. Selected individuals were recruited and seen for in-depth testing of current status. As of November 18 2020, 169 individuals at both sites had completed assessment (85% of yearly goal).

3) Significant Results.

All results are preliminary as data collection is not complete. So far, we have found the following:

Participant Characteristics: Of the 269 Tier 1 participants who have completed data collection at both sites, 40.9% are Male and 59.1% are Female. 8.2% are American Indian, 39.4% are African-American, 43.9% are Caucasian and 8.2% report being biracial. 93.7% are Non-Hispanic. Forty seven percent have never married, while 40% are married or living with a partner, 2.2% are separated and 10.4% are divorced. Sixty four percent have biological children. The largest group report completing high school (24%) or attending college (27.2%). Fifty four percent report working full time while 11.2% are disabled and 12.7% are unemployed.

Substance Use. 154 Tier 2 participants completed the Drug Checklist (10 missing) and reported a significant level of use of various substances. Of these 60.8% reported using tobacco at some time and 42.7% reported using it currently. This is well above the national average. Of those smoking, most (69%) reported using more than once a day. 86.1% reported using alcohol at some time and 72.8% described themselves as current drinkers. 55.2% of drinkers reported drinking more than once a week. Of those responding, 70.6% reported using marijuana at some time and 52.3% reported using currently. 52.4% of marijuana users reported using daily. 25.9% reported ever having used cocaine while 3.9% were using currently. 7 reported every having used amphetamines and 1.1% reported using currently. 8.8% of those responding reported ever having used methamphetamines while none reported current methamphetamine

use. 7.6% reported every having used opiates recreationally and none reported doing so currently. 5.8% of participants had GGPT results that were out of range indicating that they had excessive alcohol use.

Health problems. Initial results (N=255) of Tier 1 were analyzed to determine any differences in responses to the Health Survey based on Alcohol Exposure. The Average Age of individuals in this group was 39.53 (6.63) years for the PAE group and 38.65 (3.78) for the Controls. Significant differences were noted on a number of outcomes. Controls were more likely to report that they were in good health physically (X^2=8.93,p<.03) and mentally (X^2=14.33, p<.002) than were those who had been exposed to alcohol prenatally. The PAE group had a higher percentage of dental (X^2=22.9,p<.000) and hearing problems (X^2=8.2, p<.02). Cardiac problems were reported more frequently ("Heart problems as a Child", X^2=7.6, p<.05) as well as high blood pressure as an adult (X^2=10.42, p<.02). The PAE group reported more problems with digestion (X^2=11.5, p<.009) and regular problems with bowels (X^2=8.53, p<.04) as well as thyroid disorders (X^2=15.8, p<.001). Being prediabetic or diabetic did not reach significance. More allergies and autoimmune symptoms were identified by the PAE group, particularly Arthritis (12.6% vs. 1.2%). Having had cancer in some form was endorsed by 10 individuals with PAE (5.7%) and no controls. Significantly more in the PAE group reported having been diagnosed with a seizure disorder (X^2=8.25, p<.04). Mental Health disorders were also significantly more commonly diagnosed in the PAE group, including ADHD (X^2=11.65,p<.009), Depression (X^2=7.46,p<.06), Bipolar disorder (X^2=12.15,p<.007), and Anxiety Disorder (X^2=16.46,p<.001).

Environmental factors may account for some of these results. Individuals in the PAE group reported that they had significantly higher levels of Adverse Childhood Experiences (ACES) with items endorsed related to custody, substance use disorders by caregivers, incarceration by caregivers or family and being the victim of child abuse and neglect. (Alcohol; $F_{(2,251)}$=9.64,p<.000).

Regression models suggested that frequency of mental health problems was attributable to ACES, biological sex, Socioeconomic status and access to health care. However, examining specific mental health disorders found that Depression and Anxiety were related to environmental factors while a diagnosis of Attention Deficit, Hyperactivity Disorder was related to PAE and biological sex.

Cancer diagnosis was accounted for by PAE (X^2=7.69,p=.02), ACES (X^2=3.76,p=.03) and access to health insurance (X^2=4.49,p=.03) while diagnosis of a thyroid disorder was accounted for by PAE (X^2=10.39,p<.006) and ACES (X^2=4.87,p<.03) only.

These are preliminary findings and will be stronger if more responses are obtained that follow this same pattern. However, these results suggest both that PAE is a risk factor for health problems in midlife and that there are a number of post-natal environmental factors that contribute to these outcomes.

4) Key Outcomes and Other Achievements.

Recruitment is challenging. As was anticipated, recruitment of participants, first identified many years ago, can be a challenge. We have been able to continue to follow-up of these individuals although this has required considerable staff time in both Atlanta and Seattle. In Seattle, Dr. Grant has reported that control participants, in particular, are difficult to recruit. She planned to address this in two ways. The first strategy, to reimbursing participants who live outside Washington State, for travel expenses to Seattle was agreed to by a number of participants but could not be carried out due to COVID-19 travel restrictions. A second strategy is recruiting controls from families of FASD study participants. These are usually adoptive families many of whom had biological children or other children who were not alcohol-exposed. In addition to availability, these families are more likely to be aware of whether or not the sibling was alcohol-exposed than would individuals recruited from the community. This strategy has been implemented and appears to be successful.

Additional Questions

CIFASD4 Synergy. *Describe your project's interrelation with aims of the CIFASD consortium its other projects and the progress on those interactions.*

We continue our collaboration with Dr. Weinberg's lab both for immune studies and to allow for eventual group analyses of human data. This collaboration is maintained through frequent contact as well as, currently, quarterly inter-site conference calls among the 3 sites. When COVID permits, we will continue to

supply saliva samples for genetic testing to Indiana and 2-D photographs for evaluation to Dr. Suttie. Finally, dysmorphology examinations under Dr. Jones direction will resume when safe to do so.

Pandemic - May 2021. *If nothing changes with regard to the pandemic, what do you hope your project can achieve by May 2021 (the end of the 4th year budget period for CIFASD4) and/or what COVID-19 impacts will remain obstacles?*

The challenge of the CORVID-19 pandemic is affecting activities globally and we are not yet sure how long it will persist. Seattle was the initial site of concern in the United States and the University of Washington implemented a number of procedures designed to reduce risk. Currently, much of Washington State is "shut down" due to rising rates. Although Seattle has adapted to be able to carry out a number of activities, they are still limited in what they are able to do. In Atlanta, we have been able to return, as well, to a reduced in-person data collection process. We anticipate that we will see a reduction in participation during the rest of the grant year as a result of these concerns. We continue to do as much as possible using remote data collection. However, it is possible that due to this impediment, we may not achieve our recruitment goals. This problem arises not only due to the restriction on research activities but due to the reluctance of some of the potential participants who are concerned about infection. As many of the participants are in groups at very high risk for severe problems due to COVID, we have to respect their concerns. Nevertheless, given the rate of data collection, we should achieve our recruitment goals particularly for Tier 2. In addition, we believe that the COVID supplement will provide valuable information about the impact of this disorder on this high-risk population.

Similarly, we had anticipated working with Dr. Jones to collect dysmorphology data on our participants in Atlanta. There have been problems in doing so and we trained Dr. Ritfeld to help with this. However, due to the pandemic, this is proving a problem even with telehealth.

Despite these limitations, preliminary data analysis suggests that the results of this study will be valuable both for the information it provides and in providing directions for future research. We believe that this study is opening up new aspects in the study of the effects of PAE on health and development.

Administrative Supplements. *For this reporting period (June 2020 to present), please list any Administrative Supplement associated with this award and a sentence or two about what it entails*.

NIH/NIAAA: *Supplement to U01AA026108* (PI: Coles), Fetal Alcohol Spectrum Disorders in Adults: COVID Supplement, 10/1/20-5/31/22
This project will evaluate the impact of the COVID-19 pandemic on the adult participants in the parent grant.

NIH/NIAAA: *Supplement to U01AA026108* (PI: Coles), Neurobehavioral and Physical Health Outcomes in Offspring of Individuals with Fetal Alcohol Spectrum Disorders, 8/15/20-5/31/22
This award provides support under the Research Supplements to Promote Diversity in Health-Related Research Program for Gaby Ritfeld to improve the diversity of the research workforce by recruiting and supporting students, post-doctorates, and eligible investigators from diverse backgrounds, including those from groups that have been shown to be underrepresented in health-related research. Her project will focus on parenting practices of alcohol-affected adults and their children's outcomes.

Publications/Abstracts June 2017 - present

Publications [In Preparation & Submitted]

Kable, JA, Mehta, PK & Coles, CD. (In revision) Alterations in Insulin Levels in Adults with Prenatal Alcohol Exposure, Submitted to *Alcoholism: Clinical and Experimental Research*. (supported by grant to Dr. Kable as well as this U01)

Poster Abstracts and Presentations

Coles, CD. *Exploring an Undiscovered County: Effects of Prenatal Alcohol Exposure in Adulthood.* Keynote address for the Proof Alliance Annual Meeting, October 22, 2020

Principal Investigators: Alison Noble; Michael Suttie
Institution: University of Oxford
CIFASD4 Project: Image Analysis of Neurofacial Effects of Prenatal Alcohol Exposure
Grant Number: 5U01AA014809-17

Specific Aims

In CIFASD4, therefore, we propose to investigate the following aims:
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;
2. fetal ultrasound analysis to detect facial, cranial and neural effects of prenatal alcohol exposure with neonatal follow-up;
3. algorithm and software development to improve current analysis of face-brain-alcohol interactions.

Accomplishments

1) Major Activities.

Clinical Translation
A gratefully received admin supplement from the previous year allowed us to recruit a software engineer with industry experience, to push forward on the clinical translation of our 3D facial analysis tools. Work carried out since June 2020 has predominantly focused on packaging our 3D analysis tools into a REST API, which allows internal/external (registered) users to upload a 3D image and receive analysis feedback utilizing our tools. We are hoping this will play a role in the clinical workflow for the analysis of facial dysmorphism across the FASD spectrum.

Additionally, we are looking into new methods for early identification of FASD associated features in a neonatal population, using transfontanelle ultrasound imaging.

Prenatal Ultrasound Imaging
Earlier in the year, in collaboration with Tina Chambers, we received a number of ultrasound images from a Ukraine cohort for feature extraction and analysis. Work on these images is in progress.

2D Facial Analysis
We have continued to push analysis using 2D images of adults and those collected from the DIGFASD study. New methods for utilizing 2D images for telemedicine are also being explored.

CIFASD 5 Preparation
We feel now an important time to lay the foundations for a future application for CIFASD 5 if there is to be another phase. We have established contact with a few key personnel for access to biobanks such as PASS, to establish the state and availability of data.

2) Specific Objectives.

Clinical Translation
- Deploy FaceScreen analysis tools which utilize dense surface modelling onto an accessible and secure server
- Develop web based front end, for user access
- Develop iPhone app for interaction using Bellus3D scanning technology
- Investigate the potential for transfontanelle ultrasound for scanning

Neonatal Transfontanelle Ultrasound Imaging
- Preliminary analysis comparing corpus callosum extractions from MRI (gold standard) to transfontanelle ultrasound images on an exposed population.

Prenatal Ultrasound Imaging
- Extract facial features from images
- Build DSM model representing facial profile

2D Facial Analysis
- Generate models of facial differences from DIG FASD study, looking into potential correlations between facial dysmorphism and genetic variants from whole-exome sequencing data.

3) Significant Results and 4) Key Outcomes and Other Achievements.

Clinical Translation

This has been the main focus of our work as a group in the latter part of this year. We have successfully deployed our software onto a server (hosted by University of Oxford), which can be access through API calls. In addition, we have built a user-friendly web-based front end for users to test. In its current state, images can be uploaded directly using the web page from the large static and mobile Canfield camera, the Bellus3D iPhone app or the Bellus3D Android camera. Currently, a spare set of 16 landmarks is required for the Canfield systems, but automated Bellus3D landmarks are utilized for further refinement and model building. Once uploaded, selected facial analysis can be generated to show facial dysmorphism including, PFL, control-FAS classification testing, mid-facial flatness and an interactive 3D heatmap showing normalized difference compare to age-sex matched controls. This can also be downloaded as a pdf report. These results can be generated from an image in less than 30 seconds.

In addition, we have started to build an iPhone app using the Bellus3D software, capable of interacting with the FaceScreen server. A basic prototype is currently in early testing but we hope to have something which can be distributable to other CIFASD members before the end of the year, and we would like to demo this in the end of year meeting.

Neonatal Transfontanelle Ultrasound Imaging

We have segmented the corpus callosum from 40 neonatal US and MRI images, in an attempt to compare transfontanelle US imaging with an MRI gold standard. Corresponding MRI and US Images were collected from subjects within 2 weeks of each other, and manually segmented before building shape models to assess morphology. So far, we are seeing discrepancies which appear to be caused by the segmentation process. However, results are promising as associated alcohol-exposure differences can be seen in both MRI and US images. This is promising early result, which may pave the way for inexpensive early detection.

Prenatal Ultrasound Imaging

Using machine learning based segmentation techniques we are hoping to extract facial features from the US videos from the Ukraine subset. Currently, we are extracting facial profiles manually in an attempt to train and analyse shape differences. This work is underway, and a subset alcohol-exposed and control profiles have been extracted. The next phase will determine if we can identify shape differences to differentiate exposed and non-exposed individuals.

CIFASD 5 Preparation

We are interested in looking into placental histology, as our groups within our department have developed sophisticated machine learning tools for the identification of cellular abnormalities. Over the past few months we have been in contact with key personnel within the PASS network to investigate the possibility of obtaining sample data. Further talks are scheduled before the end of the year.

Additional Questions

CIFASD4 Synergy. *Describe your project's interrelation with aims of the CIFASD consortium its other projects and the progress on those interactions.*

Tina Chambers – Analysis of prenatal ultrasound images from a Ukrainian population image capture and transfer to produce automated measurements.

Tatiana Foroud, Leah Wetherill – We have recently been working to investigate if any facial differences are apparent from the different gene carrier groups identified from whole-exome sequencing (*KIF2A, HTT and CRIPAK)* using 2D image analysis.

Tatiana Foroud, Clare Coles and Joanne Weinberg – working with these members to collect 2D and 3D image data.

Scott Parnell, Ken Jones, Tina Chambers, Miguel Del Campo currently collaborating for infant/neonatal study where we are seeing smoke/drug-alcohol interactions.

Pandemic - May 2021. *If nothing changes with regard to the pandemic, what do you hope your project can achieve by May 2021 (the end of the 4th year budget period for CIFASD4) and/or what COVID-19 impacts will remain obstacles?*

Our university buildings remain closed as the UK enters its second national lockdown. We don't foresee this situation changing until mid-next year. However, request to access some building resources has been submitted. Childcare issues that were present in the first lockdown have been resolved in the group as childcare and schools remain open this time. Lack of office access may pose as a problem for integrating new team members into the project, we hope to overcome this with frequent video chats and scheduled weekly progress update meetings.

We hope our project can achieve the following before May 2021:
- Full deployment and clinical testing phase for FaceScreen server utilizing inputs from FaceScreen iPhone App and PC
- Automated landmarking technique integration
- Successful submission of papers in preparation
- Ultrasound facial profile analysis complete
- Fill a software developer post at Oxford to focus on clinical translation.

Administrative Supplements. *For this reporting period (June 2020 to present), please list any Administrative Supplement associated with this award and a sentence or two about what it entails.* n/a

Publications June 2017 - present

Publications [Accepted & In Press]

Fu Z, Jiao J, Suttie M, Noble A.J. Cross-task representation learning for anatomical landmark detection (2020). arXiv:2009.13635

Huang, R., Suttie, M., Noble, J.A. An automated CNN-based 3D anatomical landmark detection method to facilitate surface-based 3D facial shape analysis. In: Medical Image Computing and Computer-Assisted Intervention (MICCAI) Workshops. pp. 163–171 (2019).

Basart H, Suttie M, Ibrahim A, Ferretti P, van der Horst CMAM, Hennekam RC, Hammond P. Objectifying micrognathia using three-dimensional photogrammetric analysis. *J Craniofac Surg*. 2018 Nov;29(8):2106-2109. doi: 10.1097/SCS.0000000000005056.

Huang R, Namburete A, Noble A. Learning to segment key clinical anatomical structures in fetal neurosonography informed by a region-based descriptor. J Med Imaging (Bellingham). 2018 Jan;5(1):014007. doi: 10.1117/1.JMI.5.1.014007. PMCID: PMC5845099.

Dou X, Menkari C, Mitsuyama R, Foroud T, Wetherill L, Hammond P, Suttie M, Chen X, Chen SY, Charness ME; 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*. 2018 Mar;32(3):1364-1374. doi: 10.1096/fj.201700970. PMCID: PMC5892731.

Suttie M, Wozniak JR, Parnell SE, et al. Combined face–brain morphology and associated neurocognitive correlates in fetal alcohol spectrum disorders. *Alcohol Clin Exp Res*. 2018;42(9):1769-1782. doi:10.1111/acer.13820 PMCID: PMC6120799

Suttie M, Wetherill L, Jacobson SW, et al. 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 PMCID: PMC5563255

Publications [In Preparation & Submitted]

M Suttie, H Odendaal, Elliott AJ, S Parnell, J R. Wozniak, L Wetherill, EP Riley, T Foroud , P Hammond and the CIFASD. Facial Morphology of concurrent prenatal alcohol and smoke exposure using 3D imaging. *In preparation.*

Zeyu Fu, Jianbo Jiao, Michael Suttie, and J. Alison Noble. Regularized transfer learning for anatomical landmark detection in fetal alcohol syndrome. *In preparation.*

Principal Investigators: Kazue Hashimoto-Torii; Masaaki Torii
Institution: Children's National
CIFASD4 Project: Biomarker for Intellectual Disability in Children Prenatally Exposed to Alcohol
Grant Number: 5UH2AA026106-02

Specific Aims

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

Aim 2: Assess the biomarkers in human PAE subjects.

Accomplishments

1) Major Activities. During the second NCE, we have been working to write up a paper of results in Aim 1 study. The collection of human blood has been suspended, and thus no progress in Aim 2.

2) Specific Objectives. qRT-PCR for validation of the biomarker expressions that were originally defined by RNA sequencing in mouse study. With the defined 28 splicing biomarkers, various biological annotations were done; prediction of protein functional changes due to change of splicing isoforms (using structural biological methods), existence of non-sense mediated decay in some of the splicing biomarkers.

3) Significant Results. qRT-PCR validated former RNA seq data. Annotations of the 28 splicing biomarkers are consistent with former knowledges and relevant to the phenotypes that are predicated by those biomarkers.

4) Key Outcomes and Other Achievements. R01 was submitted by using partially the data of the UH2.

Additional Questions

CIFASD4 Synergy. *Describe your project's interrelation with aims of the CIFASD consortium its other projects and the progress on those interactions.*

Using the WGS and WES data, Tatiana and Leah's group has been working to test whether one of biomarkers that we defined with a mouse model are associated with spatial memory performance in PAE patients.

Pandemic - May 2021. *If nothing changes with regard to the pandemic, what do you hope your project can achieve by May 2021 (the end of the 4th year budget period for CIFASD4) and/or what COVID-19 impacts will remain obstacles*?

Due to sample collection suspended in Tina's cohort, UH2's second NCE ends without completion of human study in Aim 2.

Administrative Supplements. *For this reporting period (June 2020 to present), please list any Administrative Supplement associated with this award and a sentence or two about what it entails.* N/A

Publications June 2017 - present

Publications [Accepted & In Press]

Mohammad, S., Page, S.J., Wang, L., Ishii, S., Li, P., Sasaki, T., Basha, A., Salzberg, A., Quezado, Z., Imamura, F., Nishi, H., Isaka, K., Corbin, J., Liu, J., Imamura Kawasawa, Y.*, Torii, M.*, Hashimoto-Torii, K.* Kcnn2 blockade reverses learning deficits in the mouse model of Fetal Alcohol Spectrum Disorders. *Nat Neurosci*, 23(4):533-543, 2020. PMCID: PMC7131887

Publications [In Preparation]

Dutta, D.J., Sasaki, J., Yamashita, S., Banerjee, P., Bansal, A., Sasaki, T., Kawasawa I.Y., Torii, M.*, Hashimoto-Torii, K.* Alternative gene splicing events as peripheral biomarkers of motor skill learning deficits.

Principal Investigators: Thomas Blanchard; Sandra Mooney
Institutions: University of Maryland School of Medicine; University of North Carolina Nutrition Research Institute
CIFASD4 Project: Prenatal Alcohol Effects on the Gut Microbiome Contributing to Failure to Thrive and Altered Immune Function
Grant Number: 5UH2AA026109-02

Specific Aims

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.

Accomplishments

Aim 1:
Data from Microbiome Insights were re-analyzed by Dr Yang Song, bioinformatician at the University of Maryland over the past year to determine effect of ethanol exposure via liquid diet or drinking water in adult female Long Evans rats and to determine whether offspring of control or ethanol-exposed animals have a similar fecal microbiome to the dam at weaning and/or young adulthood. Main findings: Alpha diversity of the samples decreased over time, Shannon index decreased in ethanol-exposed adult female rats compared with controls, and there is variability among the groups regarding whether or not the offspring microbiome aligns with the dam's.

Aim 2:
Cytokine / chemokine data were generated earlier, however, nothing further has been done with these data at this time.

Aim 3:
Goal not met – the original application included Aim 3 to perform a fecal microbiota transfer. This was not done due to a lack of time and funding.

Other related activities:
In collaboration with Dr Susan Smith at the Nutrition Research Institute of UNC Chapel Hill we determined a metabolic signature in the plasma of pregnant mice exposed to alcohol. This is enriched in microbial-derived metabolites relative to control animals and a subset of these metabolites was found in the alcohol-exposed fetus. This work is in a paper under re-review at SciReports (An Enriched Biosignature of Gut Microbiota-Dependent Metabolites Characterizes Maternal Plasma in a Mouse Model of Fetal Alcohol Spectrum Disorder, first author Virdee).

Two ongoing studies in my lab examine behavior outcomes in rats and in mice following prenatal alcohol exposure +/- nutritional treatments. For both studies we have collected fecal pellets and plan to perform 16S analysis to identify the microbiota. One focus of these analyses will be to determine whether the microbial signature correlates with (predicts) severity of behavioral effects and/or response to treatment.

Additional Questions

CIFASD4 Synergy. *Describe your project's interrelation with aims of the CIFASD consortium its other projects and the progress on those interactions*.

Data analysis and interpretation is still underway. As interesting observations and testable hypotheses emerge, we expect to discuss findings with Drs Weinberg (focusing on cytokine / chemokine findings) and Foroud and Wetherill to determine whether there are similar outcomes in this animal model as in the human cohorts.

Pandemic - May 2021. *If nothing changes with regard to the pandemic, what do you hope your project can achieve by May 2021 (the end of the 4th year budget period for CIFASD4) and/or what COVID-19 impacts will remain obstacles?*

It is hoped that the Blanchard paper will be submitted in this timeframe.

By May 2021, we will have the mouse and rat microbiota analyzed and hope to have the bioinformatics underway.

Administrative Supplements. *For this reporting period (June 2020 to present), please list any Administrative Supplement associated with this award and a sentence or two about what it entails.* none

Publications June 2017 - present

Publications [Accepted & In Press]

none

Publications [In Preparation & Submitted]

Impact of ethanol on the fecal microbiome of the rat. [in preparation]