CIFASD4
Late Fall 2021
Progress Reports

Principal Investigator: Edward Riley
Institution: San Diego State University
CIFASD4 Project: Administrative Core of the CIFASD
Grant Number: U24 AA014811

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, individual calls to PIs, 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 to a wide audience. 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 2021 CIFASD Progress Meeting, organized and facilitated by the AdminC, was held via Zoom on partial days on June 1st, 2nd, and 8th. Project presentations from each PI focused on progress since the last semiannual meeting held in December 2020 and included updates on new findings and publications. PIs also shared ideas on how their project may expand after this iteration of CIFASD which is now in its 5th year. A discussion on potential possibilities for a CIFASD5 was held on the last day, both as a closed session with the Advisory Committee and a larger discussion with the Steering Committee as a whole. Also on June 8th, Drs. Riley and Charness held a closed meeting this NIAAA personnel, including Drs. George Koob and Patricia Powell, where they presented an update on major CIFASD4 findings to date. Preparations are underway for the Late Fall 2021 CIFASD4 Progress Meeting which will be held during select hours on December 16th and 17th via Zoom and follow a similar format to the June meeting. Project reviews by the Science Advisory Board members will be conducted after the meeting convenes and their feedback will be shared with each PI by the AdminC.

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 monthly meetings allow PIs to share and discuss new and exciting findings. Each meeting, the CIFASD Committee Chairs give monthly reports on the status of CIFASD publications at the various stages from idea conception to acceptance,

and an overview of external data requests both received and under review. The AdminC assists with the collection and summarization of data required for these committee status updates (e.g., the Google Drive Publications Policy forms). The monthly meetings are also an opportunity for the PI to invite guests to educate the group on their work. During a recent meeting, Dr. Riley invited former CIFASD PIs from Phase I, Drs. Sandra and Joe Jacobson, to present on data from their recent publication which appeared in *ACER* on the evolution of the physical phenotype of FASD from childhood through adolescence. A discussion on how physical parameters changing with age may impact the current diagnostic criteria ensued, with many PIs emailing questions to the Jacobsons following the meeting and within the Zoom chat to further debate and explore the information.

The typical promotion of CIFASD findings by Dr. Riley at various national conference and international meetings was again thwarted during this reporting period due to the ongoing pandemic. Symposium presentations at RSA in June 2021 included CIFASD studies on the role of genetics in FASD presented by Drs. Amanda Mahnke (Miranda lab), Eberhart, Parnell, and Olivia Weeks where Dr. Charness moderated the live Q&A session, and at ESBRA in September 2021 there was a session entitled, “Diagnosis and interventions in FASD: From genes to eHealth,” with Drs. Riley, Eberhart, Parnell, Suttie, and Petrenko as speakers. The ESBRA Congress was held in person in Timisoara, Romania; however, either institutions travel restrictions or personal circumstances prevented consortium investigators from being there. Due to the pandemic these presentations were virtual. Dr. Riley is hopeful to give in-person presentations on consortium findings soon. He is planning to attend the American College of Neuropsychopharmacology meeting in early December 2021 being held in San Juan, Puerto Rico, and to present at the Alcoholism and Stress meeting in May 2023 in Volterra, Italy. Drs. Charness and Riley are currently recruiting and working on assembling CIFASD-themed symposia submissions for the ISBRA/ESBRA 2022 and EUFASD 2022 meetings. Another avenue Dr. Riley continues to promote CIFASD findings is by serving (since 2020) as an expert for a Psychwire ASK online Q&A on FASD. He responds to submitted questions and posts his answers.

In anticipation of an RFA for the continuation of CIFASD, in mid-April 2021 the AdminC announced an open call for projects via listserv emails and postings through several organizations: RSA, the FASD Study Group, ISBRA, BDRP, ISDP, NADIA, DEARC, NCANDA, and NIH MHHD. The PI also requested advice from Dr. J. Arroyo from NIAAA-MHHD, for suggestions on other recruitment avenues to pursue. Current CIFASD investigators were invited to respond and to encourage others to respond as well, and a special emphasis was advertised on issues involving and addressing diversity. Thirty one-page letters of intent were received by late April 2021. The letters of intent were reviewed and ranked by members of the CIFASD Advisory Committee (AdminC personnel, SAB members and the NIAAA Project Scientist) based on their scientific merit and fit within a consortium framework. After numerous Zoom meetings and lengthy and in-depth discussions, 20 proposals were invited to submit an expanded 5-page proposal by early June. These expanded proposals were reviewed and ranked by members of the Advisory Committee and external reviewers not affiliated with CIFASD. External reviewers were selected by the PI and their names were blinded from the rest of the Advisory Committee and the proposal PIs. The Advisory Committee held final deliberations, suggested revisions to aims, addressed budgetary issues, and identified potential project interactions over numerous Zoom meetings in June and July with individual prospective PIs and the pool as a whole. Each PI moving into the final phase was provided a description of every project still in the running and an Excel file with the proposed measures for each project, which also included suggestions for interactions and common measures among projects from the Advisory Committee. All were also provided with the feedback from the external reviewers. From the 20 proposals, three U24 Resources (requested by the RFAs), eight U01 projects, and two UH2 exploratory/developmental projects were selected for inclusion in the CIFASD5 renewal. Research plans and budgets were submitted to the AdminC personnel for review and edits prior to submission from each PI to NIH. The PI and NIAAA Project Scientist held meetings to discuss the budgets and the cap provided in the RFAs. All proposals were submitted by August 16, 2021 and are currently under review. The AdminC collected and distributed the Specific Aims and Research Strategies for each of the 13 proposed CIFASD5 projects and shared with all prospective PIs and members of the AdminC and SAB to prepare for the reverse site visit (RSV). The AdminC has requested investigators read the proposals and compose questions potential reviewers may ask and will be planning a practice/mock RSV once the dates of the actual RSV are announced.

This fall Dr. Riley has been helping CIFASD4 PIs navigate challenges stemming from the ongoing pandemic and its effects on their project’s timeline. He interfaces with the PIs and NIAAA advisors regularly to determine the best paths forward for the project and the consortium as a whole. These efforts are particularly critical due to the pending renewal for CIFASD5. Of note with regard to NIAAA advisors, this fall Dr. Bill Dunty stepped down from his role as the Project Scientist for CIFASD at NIAAA; this role has now been filled by Dr. Elizabeth Powell.

Dr. Chockalingam, through the Blue Resonance, LLC subaward, has continued to support Dr. Mattson with her BRAIN-online neurobehavioral data collection and FASD decision tree applications. He routinely provides support, bug fixes and backend maintenance for the systems. He has also been assisting the Dysmorphology Core with their efforts in Alaska and has provided support and training for health care providers utilizing the MorpheusQ smartphone technologies during their FASD screenings. A successful spin-off of this work was funded this fall from NIAAA with an R21 entitled, "Smartphone-based application to assist in the screening/diagnosis of FASD."

Ten new publications citing CIFASD funding have been published since the June 2021 progress meeting, bringing the total number of publications citing CIFASD grants as a source of support in 2021 to 25, and the overall total number of publications since the consortium's inception to 324. With regard to the AdminC, the grant has been cited as a source of support in 114 papers, 40 since the start of CIFASD4, including 10 in 2021.

The AdminC continues to access and monitor the allocation of shared consortium resources, such as the portable Canfield Vectra 3D cameras, a Bellus3D mobile phone camera, iPhones, and tablets. It also maintains the CIFASD.org website and updates news items as received.

The FFR for the budget year ending 5/31/2021 was accepted by NIAAA on 10/6/2021 and a carryover request was submitted for consideration by the AdminC on 10/14/2021 and is currently under review.

Additional Questions

Final Year. What do you plan to do between now and May 2022* to accomplish your CIFASD4 goals and objectives? *For those with UH2s funded in 2021, what are your goals for year two of your support?

The AdminC will continue to support and encourage the publication of CIFASD4 findings and will coordinate the logistics for the CIFASD5 reverse site visit in 2022.

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.

Administrative Supplements. For this reporting period (June 2021 to present), please list any Administrative Supplement associated with this award and a sentence or two about what it entails and its current status.

n/a

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

Publications [Accepted & In Press]

Bernes GA, Courchesne NS, Hyland MT, Villodas MT, Coles CD, Kable JA, May PA, Kalberg WO, Sowell ER, Wozniak JR, Jones KL, **Riley EP**, Mattson SN; CIFASD. Development and validation of a postnatal risk score that identifies children with prenatal alcohol exposure. *Alcohol Clin Exp Res*., 2021, In press. PMCID: PMC Journal - In Process

Rodriguez CI, Vergara VM, Calhoun VD, Savage DD, Hamilton DA, Tesche CD, Stephen JM. Disruptions in global network segregation and integration in adolescents and young adults with fetal alcohol spectrum disorder. *Alcohol Clin Exp Res.*, 2021 Sep;45(9):1775-1789. PMCID: PMC Journal - In Process

Roos A, Wedderburn CJ, Fouche JP, Subramoney S, Joshi SH, Woods RP, Zar HJ, Narr KL, Stein DJ, Donald KA. Central white matter integrity alterations in 2-3-year-old children following prenatal alcohol exposure. *Drug Alcohol Depend*., 2021 Aug 1;225:108826. PMCID: PMC8299546

Sidik A, Dixon G, Buckley DM, Kirby HG, Sun S, Eberhart JK. Exposure to ethanol leads to midfacial hypoplasia in a zebrafish model of FASD via indirect interactions with the Shh pathway. *BMC Biol*., 2021 Jul 1;19(1):134. PMCID: PMC8247090

Roos A, Fouche JP, Ipser JC, Narr KL, Woods RP, Zar HJ, Stein DJ, Donald KA. Structural and functional brain network alterations in prenatal alcohol exposed neonates. *Brain Imaging Behav*., 2021 Apr;15(2):689-699. PMCID: PMC7572489

Bernes GA, Villodas M, Coles CD, Kable JA, May PA, Kalberg WO, Sowell ER, Jones KL, **Riley EP**, Mattson SN; CIFASD. Validity and reliability of executive function measures in children with heavy prenatal alcohol exposure: Correspondence between multiple raters and laboratory measures. *Alcohol Clin Exp Res*., 2021 Mar;45(3):596-607. PMCID: PMC7969422

de Water E, Rockhold MN, Roediger DJ, Krueger AM, Mueller BA, Boys CJ, Schumacher MJ, Mattson SN, Jones KL, Lim KO, Wozniak JR. Social behaviors and gray matter volumes of brain areas supporting social cognition in children and adolescents with prenatal alcohol exposure. *Brain Res*., 2021 Feb 20:147388. PMCID: PMC8377082.

Rockhold MN, Krueger AM, de Water E, Lindgren CW, Sandness KE, Eckerle JK, Schumacher MJ, Fink BA, Boys CJ, Carlson SM, Fuglestad AJ, Mattson SN, Jones KL, **Riley EP**, Wozniak JR. Executive and social functioning across development in children and adolescents with prenatal alcohol exposure. *Alcohol Clin Exp Res*., 2021 Feb;45(2):457-469. PMCID: PMC7887051

Roediger DJ, Krueger AM, de Water E, Mueller BA, Boys CA, Hendrickson TJ, Schumacher MJ, Mattson SN, Jones KL, Lim KO, Wozniak JR. Hippocampal subfield abnormalities and memory functioning in children with fetal alcohol Spectrum disorders. *Neurotoxicol Teratol*., 2021 Jan-Feb;83:106944. PMCID: PMC7855420

Moore EM, Glass L, Infante MA, Coles CD, Kable JA, Jones KL, **Riley EP**, Mattson SN. Cross-sectional analysis of spatial working memory development in children with histories of heavy prenatal alcohol exposure, *Alcohol Clin Exp Res*., 2021 Jan;45(1):215-223. PMCID: PMC7875477

Wedderburn CJ, Subramoney S, Yeung S, Fouche JP, Joshi SH, Narr KL, Rehman AM, Roos A, Ipser J, Robertson FC, Groenewold NA, Gibb DM, Zar HJ, Stein DJ, Donald KA. Neuroimaging young children and associations with neurocognitive development in a South African birth cohort study, *Neuroimage.*, 2020 Oct 1;219:116846. PMCID: PMC7443699

Krueger AM, Roediger DJ, Mueller BA, Boys CA, Hendrickson TJ, Schumacher MJ, Mattson SN, Jones KL, **Riley EP**, Lim KO, Wozniak JR. Para-limbic structural abnormalities are associated with internalizing symptoms in children with prenatal alcohol exposure, *Alcohol Clin Exp Res*., 2020 Aug;44(8):1598-1608. PMCID: PMC7484415

Sullivan EV, Moore EM, Lane B, Pohl KM, **Riley EP**, Pfefferbaum A. Graded cerebellar lobular volume deficits in adolescents and young adults with fetal alcohol spectrum disorders (FASD), *Cereb Cortex,* 2020 Jul 30;30(9):4729-4746. PMCID: PMC7391273

Inkelis SM, Moore EM, Bischoff-Grethe A, **Riley EP**. Neurodevelopment in adolescents and adults with fetal alcohol spectrum disorders (FASD): A magnetic resonance region of interest analysis, *Brain Res*., 2020 Apr 1;1732:146654. PMCID: PMC7067519

Dou X, Lee JY, **Charness ME**. Neuroprotective peptide NAPVSIPQ antagonizes ethanol inhibition of L1 adhesion by promoting the dissociation of L1 and ankyrin-G*, Biol Psychiatry*, 2020 Apr 1;87(7):656-665. PMCID: PMC7056560

Kable JA, Coles CD, Mattson SN. Neurodevelopmental outcomes associated with prefrontal cortical deoxygenation in children with fetal alcohol spectrum disorders, *Dev Neuropsychol*., 2020 Jan-Feb;45(1):1-16. PMCID: PMC7080191

Swartz ME, Lovely CB, McCarthy N, Kuka T, Eberhart JK. Novel ethanol-sensitive mutants identified in an F3 forward genetic screen, *Alcohol Clin Exp Res*., 2020 Jan;44(1):56-65. PMCID: PMC6980918

Bodnar TS, Raineki C, 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**. Immune network dysregulation associated with child neurodevelopmental delay: Modulatory role of prenatal alcohol exposure, *J Neuroinflammation*, 2020 Jan 28;17(1):39. PMCID: PMC6988366

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. PMCID: PMC6722014

Donald KA, Wedderburn CJ, Barnett W, Nhapi RT, Rehman AM, Stadler JAM, Hoffman N, Koen N, Zar HJ, Stein DJ. Risk and protective factors for child development: An observational South African birth cohort, *PLoS Med.*, 2019 Sep 27;16(9):e1002920. PMCID: PMC6764658

Wozniak JR, **Riley EP**, **Charness ME**. Clinical presentation, diagnosis, and management of fetal alcohol spectrum disorder, *Lancet Neurol*., 2019 Aug;18(8):760-770. PMCID: PMC6995665

Doyle LR, Coles CD, Kable JA, May PA, Sowell ER, Jones KL, **Riley EP**, Mattson SN; **CIFASD**. Relation between adaptive function and IQ among youth with histories of heavy prenatal alcohol exposure, *Birth Defects Res*., 2019 Jul 15;111(12):812-821. PMCID: PMC6650363

Doyle LR, Glass L, Wozniak JR, Kable JA, **Riley EP**, Coles CD, Sowell ER, Jones KL, Mattson SN; **CIFASD**. Relation between oppositional/conduct behaviors and executive function among youth with histories of heavy prenatal alcohol exposure, *Alcohol Clin Exp Res*., 2019 Jun;43(6):1135-1144. PMCID: PMC6551300

Barrett CE, Kable JA, Madsen TE, Hsu CC, Coles CD. The use of functional near-infrared spectroscopy to differentiate alcohol-related neurodevelopmental impairment, *Dev Neuropsychol*., 2019 Mar-Apr;44(2):203-219. PMCID: PMC6423538

Gangisetty O, Sinha R, Sarkar DK. Hypermethylation of proopiomelanocortin and period 2 genes in blood are associated with greater subjective and behavioral motivation for alcohol in humans, *Alcohol Clin Exp Res*., 2019 Feb;43(2):212-220. PMCID: PMC6370509

Subramoney S, Eastman E, Adnams C, Stein DJ, Donald KA. The early developmental outcomes of prenatal alcohol exposure: A review, *Front Neurol*., 2018 Dec 18;9:1108. PMCID: PMC6305542

Doyle LR, Moore EM, Coles CD, Kable JA, Sowell ER, Wozniak JR, Jones KL, **Riley EP**, Mattson SN; **CIFASD**. Executive functioning correlates with communication ability in youth with histories of heavy prenatal alcohol exposure, *J Int Neuropsychol Soc*., 2018 Nov;24(10):1026-1037. PMCID: PMC6237635

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. PMCID: PMC6344127

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. PMCID: PMC6120799

Charness ME. The adolescent brain cognitive development study external advisory board, *Dev Cogn Neurosci*., 2018 Aug;32:155-160. PMCID: PMC6969232

Gross LA, Moore EM, Wozniak JR, Coles CD, Kable JA, Sowell ER, Jones KL, **Riley EP**, Mattson SN; **CIFASD**. Neural correlates of verbal memory in youth with heavy prenatal alcohol exposure, *Brain Imaging Behav*., 2018 Jun;12(3):806-822. PMCID: PMC5745322

Donald KA, Hoogenhout M, du Plooy CP, Wedderburn CJ, Nhapi RT, Barnett W, Hoffman N, Malcolm-Smith S, Zar HJ, Stein DJ. Drakenstein Child Health Study (DCHS): Investigating determinants of early child development and cognition, *BMJ Paediatr Open*, 2018 Jun 13;2(1):e000282. PMCID: PMC6014194

Fernandes Y, Buckley DM, Eberhart JK. Diving into the world of alcohol teratogenesis: A review of zebrafish models of fetal alcohol spectrum disorder, *Biochem Cell Biol*., 2018 Apr;96(2):88-97. PMCID: PMC7413215

Hendrickson TJ, Mueller BA, Sowell ER, Mattson SN, Coles CD, Kable JA, Jones KL, Boys CJ, Lee S, Lim KO, **Riley EP**, Wozniak JR. Two-year cortical trajectories are abnormal in children and adolescents with prenatal alcohol exposure, *Dev Cogn Neurosci*., 2018 Apr;30:123-133. PMCID: PMC5949095

Dou X, Menkari C, Mitsuyama R, Foroud T, Wetherill L, Hammond P, Suttie M, Chen X, Chen SY, **Charness ME**; **CIFASD**. 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. PMCID: PMC5892731

Kable JA, Coles CD; **CIFASD**. Prefrontal cortical responses in children with prenatal alcohol-related neurodevelopmental impairment: A functional near-infrared spectroscopy study, *Clin Neurophysiol*., 2017 Nov;128(11):2099-2109. PMCID: PMC5675790

Infante MA, Moore EM, Bischoff-Grethe A, Tapert SF, Mattson SN, **Riley EP**. Altered functional connectivity during spatial working memory in children with heavy prenatal alcohol exposure, *Alcohol*, 2017 Nov;64:11-21. PMCID: PMC5635832

Wozniak JR, Mueller BA, Mattson SN, Coles CD, Kable JA, Jones KL, Boys CJ, Lim KO, **Riley EP**, Sowell ER; **CIFASD**. Functional connectivity abnormalities and associated cognitive deficits in fetal alcohol Spectrum disorders (FASD), *Brain Imaging Behav*., 2017 Oct;11(5):1432-1445. PMCID: PMC5389933

Taggart TC, Simmons RW, **Thomas JD**, **Riley EP**. Children with heavy prenatal alcohol exposure exhibit atypical gait characteristics, *Alcohol Clin Exp Res*., 2017 Sep;41(9):1648-1655. PMCID: PMC5581268

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. PMCID: PMC5563255

Principal Investigator: Kenneth Lyons Jones
Institution: University of California, San Diego
CIFASD4 Project Title: Dysmorphology Research Resource
Grant Number: U24 AA014815

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

Specific Aim #2: To further develop and refine the telemedicine approach developed in the last funding period, we will expand upon our telemedicine capabilities in order to
1) reach children in underserved areas that lack access to physicians with expertise in clinical recognition of FASD,
2) train additional physicians in the clinical identification of FASD and
3) recruit additional research subjects who have been prenatally exposed to alcohol.

For this specific aim the Dysmorphology Research Resource will:
•Continue to work in collaboration with Dr. Jeff Wozniak at the University of Minnesota as well as with the Minnesota Organization on Fetal Alcohol Syndrome: MOFAS to improve upon the telemedicine program developed and field tested by the Dysmorphology Research Resource in the last funding period.
•Expand the telemedicine program in Minnesota and establish a new telemedicine program in New Mexico to diagnose FAS at Indian Health Hospitals and Clinics in remote areas throughout the state.
•Identify patients prenatally exposed to alcohol that may be eligible for participation in the CIFASD registry and refer to the registry, once
functional.

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

Accomplishments

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.

1) Major Activities. Between June 2021 and December 2021, using the CIFASD physical examination protocol and classification system, Neither Dr Jones nor Dr. del Campo performed any face-to-face physical examinations in any of the CIFASD clinical sites However Dr. Jones trained via telemedicine Dr. Zvii. -, a postdoctoral fellow

in psychiatry at Emory working with Dr. Coles and Kable to perform physical examinations to diagnose FAS. In addition 5 normal adults were examined face-to-face and by the Morpheus Q app to validate the Morpheus Q app and the Morpheus Q app was used to measure palpebral fissure length, as well as configuration of the philtrum and vermilion border on telemedicine facial images in 3 children equal to or less that 3 years of age.

2) Specific Objectives. To validate the use of telemedicine, we have begun using telemedicine ourselves to diagnose children with FASD as well as to train pediatricians on how to diagnose FASD and to re-train pediatricians on diagnosis of FASD.

3) Significant Results. Dr. Jones and Dr. del Campo performed physical examinations by telemedicine at the UCSD/RADY Children's Hospital FASD Clinic on an average of 4 children per week who had been prenatally exposed to alcohol. In addition, we trained face-to-face 12 pediatric residents on how to perform a physical examination to rule out FAS.

4) Key Outcomes and Other Achievements. Drs. del Campo and Jones have trained 12 Pediatric Residents and Fellows who previously had no prior knowledge regarding the performance of a physical examination on children with and without fetal alcohol syndrome. Furthermore we have developed experience in training physicians and other healthcare providers using telemedicine and have used and validated the morpheus Q app in different settings to validate its effectiveness.

Specific Aim #2: To further develop and refine the telemedicine approach developed in the last funding period, we will expand upon our telemedicine capabilities

1) Major Activities. In previous reporting periods, we demonstrated high convergent Validity of Telemedicine, and we performed training via Telemedicine in several settings. We have continued to evaluate patients exposed to alcohol via telemedicine and have successfully performed accurate physical exams in the clinical and research settings remotely following the procedures described in our previous paper. Del Campo M, et al. Use of Telemedicine for the Physical Examination of Children With Fetal Alcohol Spectrum Disorders. Alcohol Clin Exp Res. 2021 Feb;45(2):409-417. doi: 10.1111/acer.14533.

2) Specific Objectives. To provide training via telemedicine to providers in underserved areas. In collaboration with Ganz Chockalingam we have trained 6 groups of providers in Alaska via telemedicine on physical examination using standard tools (i.e. ruler and lip-philtrum guide) and using Morpheus Q. We have had additional meetings to troubleshoot the use of the App and ensure correct use.

3) Significant Results. Accurate examination and training via telemedicine were successfully achieved.

4) Key Outcomes and Other Achievements. TM examinations for the identification of physical features of FASD have been proven to be feasible, valid and reliable, and have very high sensitivity and specificity for the detection of these features.

Specific Aim #3: Contribute to the CIFASD Consortium research studies by utilizing and expanding upon the San Diego FASD research subject pool established at UCSD/Rady Children's Hospital

1) Major Activities. The purpose of the research subject pool is to ascertain children with prenatal alcohol exposure whose parents or guardians can be contacted to participate in CIFASD research projects. Parents or guardians of children prenatally exposed to alcohol who consent to being part of the FASD Research Subject Pool will be re-contacted for research studies if their child meets inclusion criteria. This FASD Research Subject Pool has provided an invaluable source of potential research subjects to support the CIFASD Consortium's research projects and its overall mission.

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

3) Significant Results. Between June 2021 and December 2021, through the FASD Clinic at RCHSD, we have enrolled 7 new subjects (2 males, 5 females) into the FASD Research Subject Pool bringing our total registry numbers up to 278. In addition, we have referred 8 subjects from this research pool to Dr. Sarah Mattson's Decision Tree project.

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

Additional Questions

Final Year.

- Between now and May 2022 plan we plan to train an additional 25 physicians and other health care providers to make a diagnosis of FASD using telemedicine in order to increase the number of providers who can diagnose FAS. We will make a major effort to do this in underserved areas of the U.S. where only a very small number of health care providers are capable of making the diagnosis.
- We will attempt to increase our visibility in Alaska, a state where the prevalence of this disorder is extremely high.
- Hopefully over the next 6 months we will be able to refer again the same number of subjects that we were able to refer to Dr. Sarah Mattson's FASD Decision Tree prior to the pandemic.
- Hopefully we will be able to enlist into the FASD Research Repository an equal number of subjects as we were prior to the pandemic.

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

- The Dysmorphology Research Resource was established primarily to 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. (see Specific Aim #1). However, over the last 6 months the majority of clinical sites have not been recruiting new subjects. Therefore, the extent of CIFASD4 synergy has decreased over the last 6 months.
- Through the Dysmorphology Research Registry's proposed project in Alaska, it is hoped that telemedicine will provide the opportunity to provide not only physical diagnosis of FASD but neuropsychological evaluation, interventions, support and therapy programs for children with FASD and their families in an underserved area.
- Through the Research Registry at UCSD/Rady Children's Hospital, we will hopefully be able to provide subject records, and specimens to various research projects being conducted by researchers throughout the U.S. and Canada.

Administrative Supplements. For this reporting period (June 2021 to present), please list any Administrative Supplement associated with this award and a sentence or two about what it entails and its current status

3U24AA014815-16S1 Dysmorphology Research Resource Supplement: Aim 1. To meet the study-specific research needs of the CIFASD consortium by facilitating San Diego FASD Research Subject Pool participation in CIFASD projects. To accomplish this aim, we will: a. Screen, recruit, and consent FASD Research Subject Pool participants to CIFASD consortium studies. b. Coordinate the collection of the data required for each given CIFASD consortium study. Biorepository collections have been halted since March 2020 due to the COVID-19 pandemic; thus, no new samples were collected during the reporting period.

Publications/Abstracts

Publications [Accepted & In Press]

Bernes GA, Villodas M, Coles CD, Kable JA, May PA, Kalberg WO, Sowell ER, Jones KL, Riley EP, Mattson SN. Validity and Reliability of Executive Function Measures in Children With Heavy Prenatal Alcohol Exposure: Correspondence Between Multiple Raters and Laboratory Measures. Alcoholism, clinical and experimental research. 2021 March;45(3):596-607. PubMed PMID: 33433001; DOI: 10.1111/acer.14547.

de Water E, Rockhold MN, Roediger DJ, Krueger AM, Mueller BA, Boys CJ, Schumacher MJ, Mattson SN, **Jones KL**, Lim KO, Wozniak JR. Social behaviors and gray matter volumes of brain areas supporting social cognition in children and adolescents with prenatal alcohol exposure. Brain Res. 2021 Feb 20;:147388. doi: 10.1016/j.brainres.2021.147388. [Epub ahead of print] PMID: 33621483

Rockhold MN, Krueger AM, de Water E, Lindgren CW, Sandness KE, Eckerle JK, Schumacher MJ, Fink BA, Boys CJ, Carlson SM, Fuglestad AJ, Mattson SN, **Jones KL**, Riley EP, Wozniak JR. Executive and Social

Functioning Across Development in Children and Adolescents With Prenatal Alcohol Exposure. Alcohol Clin Exp Res. 2021 Feb;45(2):457-469. doi: 10.1111/acer.14538. Epub 2021 Jan 22. PMID: 33349933

Moore EM, Glass L, Infante MA, Coles CD, Kable JA, Jones KL, Riley EP, Mattson SN. Cross-Sectional Analysis of Spatial Working Memory Development in Children with Histories of Heavy Prenatal Alcohol Exposure. Alcoholism, clinical and experimental research. 2021 January;45(1):215-223. PubMed PMID: 33190244; PubMed Central PMCID: PMC7875477; DOI: 10.1111/acer.14506.

Roediger DJ, Krueger AM, de Water E, Mueller BA, Boys CA, Hendrickson TJ, Schumacher MJ, Mattson SN, **Jones KL**, Lim KO, Wozniak JR. Hippocampal subfield abnormalities and memory functioning in children with fetal alcohol Spectrum disorders. Neurotoxicol Teratol. 2021 Jan-Feb;83:106944. doi: 10.1016/j.ntt.2020.106944. PMID: 33232797

Del Campo M, Beach D, Wells A, Jones KL. Use of Telemedicine for the Physical Examination of Children With Fetal Alcohol Spectrum Disorders. Alcohol Clin Exp Res. 2021 Feb;45(2):409-417. doi: 10.1111/acer.14533. Epub 2021 Feb 8. PMID: 33316074.

Krueger AM, Roediger DJ, Mueller BA, Boys CA, Hendrickson TJ, Schumacher MJ, Mattson SN, Jones KL, Riley EP, Lim KO, Wozniak JR. Para-limbic Structural Abnormalities Are Associated With Internalizing Symptoms in Children With Prenatal Alcohol Exposure. Alcohol Clin Exp Res. 2020 Aug;44(8):1598-1608. doi: 10.1111/acer.14390. Epub 2020 Jul 1. PMID: 32524616; PMCID: PMC7484415.

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 Oct;44(10):2045-2052. doi: 10.1111/acer.14430. Epub 2020 Sep 6. PMID: 32772389; PMCID: PMC7722075.

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., & 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. https://doi.org/10.1111/acer.14036

Doyle, L. R., Coles, C. D., Kable, J. A., May, P. A., Sowell, E. R., Jones, K. L., Riley, E. P., Mattson, S. N., & CIFASD (2019). Relation between adaptive function and IQ among youth with histories of heavy prenatal alcohol exposure. *Birth defects research*, *111*(12), 812–821. https://doi.org/10.1002/bdr2.1463

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., & CIFASD (2018). Executive Functioning Correlates With Communication Ability in Youth With Histories of Heavy Prenatal Alcohol Exposure. *Journal of the International Neuropsychological Society : JINS*, *24*(10), 1026–1037. https://doi.org/10.1017/S1355617718000772

Coles CD, Kable JA, Granovska IV, Pashtepa AO, Plotka LD, Dolhov VB, Wertelecki W, **Jones KL**, Chambers CD; CIFASD. Gestational age and socioeconomic status as mediators for the impact of prenatal alcohol exposure on development at 6 months. *Birth Defects Res*. 2019 Jul 15;111(12):789-796. doi: 10.1002/bdr2.1408. Epub 2018 Oct 31.

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., Foroud, T., Hammond, P., & CIFASD (2018). Combined Face-Brain Morphology and Associated Neurocognitive Correlates in Fetal Alcohol Spectrum Disorders. *Alcoholism, clinical and experimental research*, *42*(9), 1769–1782. https://doi.org/10.1111/acer.13820

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., & CIFASD (2018). Neural correlates of verbal memory in youth with heavy prenatal alcohol exposure. *Brain imaging and behavior*, *12*(3), 806–822. https://doi.org/10.1007/s11682-017-9739-2

Hendrickson TJ, Mueller BA, Sowell ER, Mattson SN, Coles CD, Kable JA, **Jones KL**, Boys CJ, Lee S, Lim KO, Riley EP, Wozniak JR. Two-year cortical trajectories are abnormal in children and adolescents with prenatal alcohol exposure. *Dev Cogn Neurosci*. 2018 Apr; 30:123-133. doi: 10.1016/j.dcn.2018.02.008. Epub 2018 Feb 21.

Publications [In Preparation & Submitted] - None

Poster Abstracts and Presentations

Doyle, L.R., Chambers, C.D., Jones, K.L., Mattson, S.N., the CIFASD. “Validation of a Decision Tree for Clinical Identification of Children Affected by Prenatal Alcohol Exposure in a Low-Risk Sample.” Presented at the 41st Annual Scientific Meeting of the Research Society on Alcoholism, San Diego, CA (June 2018) Published: Alcoholism: Clinical and Experimental Research 42(S1):115A, 2018.

Coles, C.D., Kable, J.A., Mesa, D.A., Coleman, T.P., Jones, K.L., Yevtushok, L., Kulikovsky, Y., Wertelecki, W., Chambers, C.D. “Early Identification of Effects of Prenatal Alcohol Exposure: Infant Cardiac Orienting Response as a Biomarker.” Presented at the 41st Annual Scientific Meeting of the Research Society on Alcoholism, San Diego, CA (June 2018) Published: Alcoholism: Clinical and Experimental Research 42(S1), 2018.

Principal Investigator: Christina Chambers
Institution: University of California, San Diego
CIFASD4 Project Title: Early Predictors of FASD in Ukraine
Grant Number: U01 AA014835

Specific Aims

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

Aim 1. Develop a panel of prenatal/infancy biomarkers that can predict FASD

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

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

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

Aim 3. Collaborate with others in the CIFASD consortium

Provide data and biological samples from the Ukraine cohort, along with analytical support to U01 PI Weinberg to assess markers of inflammation in mothers and their children who are or are not affected by FASD; provide data from the Ukraine cohort to U01 PI Mattson to test/adapt the FASD Decision Tree; provide data and biological samples from the Ukraine cohort and assist in the analyses for UH2 PI Torri to test the value of single cell technology in identifying specific epigenetic markers in infants/children with neurobehavioral impairment associated with prenatal alcohol exposure; provide 2D ultrasound fetal facial profiles and associated clinical data from the
Ukraine cohort to U01 PI Suttie/Noble 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.

- We have recruited 11 additional pregnant women for a total of 167 out of the goal of 200 in CIFASD 4
- In October, 2021, we shipped 21 plasma samples from Ukrainian children with matched maternal samples to Dr. Miranda's and Dr. Weinberg's labs for analysis of miRNAs and cytokines.
- In November, 2021, we received a final shipment from Ukraine of 25 new maternal plasma samples and 7 new child plasma samples; these are being distributed to Dr. Miranda and Dr. Weinberg.
- In November, 2021, the two sites in Ukraine completed data entry for the school age sample for 89 participants, and these data were analyzed by Dr. Coles.
- In October, 2021, whole genome sequencing under the XO funding opportunity was completed by the contractor, Hudson Alpha. In November, 2021, the associated clinical data for those samples was uploaded to the data tracker. Release of the sequence data to UCSD awaits approval by the contractor of the clinical data files.

2) Specific Objectives.

Enrollment and data collection activities to date are summarized in Table 1.

Table 1. Data Collection vs. Goal, Ukraine November, 2021

Visit	Enrolled/Data Capture	Goal
Pregnant women/blood samples	167	200
Ultrasounds in pregnancy	157	200
Testing 6 mo old infants	35	100
Testing 12 mo old infants	11	100
Blood samples infants	10	140
Testing school-age children	101	150
Blood samples children	58	70
3D images one site	41	65

3) Significant Results.

Aim 1a. Dr. Miranda's lab received and begun processing an additional 21 samples from Ukrainian children, age 2-3.5 yo. This will increase the sample size of our child assessment to 78, including 57 child samples that have already been assayed for circulating miRNAs within plasma. These additional samples also increase the number of children in our miRNA assessment who were also assayed for cytokines (Dr. Joanne Weinberg's group), with a total of 40 children that will be assayed for both circulating miRNAs and cytokines. We will also be able to assess miRNAs and cytokines as a collective set of biomarkers in maternal samples. We have previously published miRNA data from 93 maternal samples, collected at mid and/or late pregnancy (PMC5102408, PMC7488011). We have received an additional 30 maternal samples, resulting in a total of 123 maternal samples. These additional samples have substantial overlap with the maternal cytokine data (97 samples). Our maternal and child samples also contain 56 maternal/child pairs which can be assessed to discover novel circulating miRNA-related pathways that are conserved across mother and child following alcohol exposure during pregnancy/the *in utero* period. This work is in process now. In related work, Dr. Miranda's lab is following up on our previous work that identified 11 gestationally elevated maternal circulating miRNAs ($_{HEa}$miRNAs) that predicted infant growth deficits following prenatal alcohol exposure and regulated epithelial–mesenchymal transition in the placenta. Subsequent work now shows that a single intravascular administration of pooled murine-expressed $_{HEa}$miRNAs to pregnant mice on gestational day 10 (GD10) attenuates umbilical cord blood flow throughout gestation, resulting in a ~14.3% decrease in the Velocity-Time Integral (*VTI*, in mm^3/sec) in the umbilical artery of fetuses prenatally exposed to $_{HEa}$miRNAs on GD18. Correlational analyses correcting for prenatal exposure status shows $_{HEa}$miRNAs mediate the relationship between umbilical cord blood flow and fetal growth parameters of crown-rump length, snout-occipital distance, and philtrum length. RNAseq of the non-decidual portion of the placenta demonstrated this single exposure to $_{HEa}$miRNAs has lasting transcriptomic changes that results in upregulation of members of the Notch pathway (*Dll4*, *Rfng*, *Hey1*), which is a pathway important for trophoblast migration and differentiation. Weighted gene co-expression network analysis also identified chemokine signaling, which is responsible for regulating immune cell-mediated angiogenesis in the placenta, as an important factor for fetal growth and head size. Our data suggest that $_{HEa}$miRNAs perturb expression of placental genes relevant for angiogenesis, resulting in impaired umbilical cord blood flow and subsequently IUGR.

Aim 2a. The analysis for the School Age testing battery administered for children in the cohort originally recruited prenatally and now 7-10 years of age was completed. As shown in Table 2, significant findings were still measurable in these children consistent with prenatal alcohol dose, after adjustment for SES, age, sex, and site. No significant effects were noted on WISC Spatial Span Forward or Backward, Tema Math Score, Recall of Objects, Digit Span Forward or Backward, Speed of Information Processing, or Rapid Naming.

Table 2. Models of School Age Outcomes by Alcohol Dose at Conception and in Most Recent Two Weeks Mid-Pregnancy

Measure	N	ABO at Conception p-value	ABO Mid-Pregnancy p-value
BRIEF Global Exec Functioning Score	74	0.021	0.084
BRIEF Inhibit	74	0.012	0.009
BRIEF Working Memory	74	0.051	
BRIEF Behavioral Regulation	74	0.008	0.134
BRIEF Metacognitive Index	74	0.042	0.170
Beery VMI	74	0.019	0.076

CBCL Total Problem Score	56	0.008	0.003
CBCL Externalizing	56	0.009	0.046
CBCL Internalizing	56	0.060	0.004
CBCL Anxiety/Depression	56	0.033	0.008
CBCL Withdrawn/Depressed	56	0.314	0.049
CBCL Thought Problems T-score	56	0.002	0.011
CBCL Somatic Complaints T-score	56	0.014	0.001
CBCL Attention Problems T-score	56	0.034	0.053
CBCL Rule Breaking Behavior	56	<0.001	0.076
CBCL Aggressive Behavior	56	0.062	0.060
DAS-II Recall of Designs	89	0.027	0.010
DAS-II Nonverbal Reasoning Standard Score	89	0.576	0.715
DAS-II Spatial Standard Score	89	0.086	0.012
DAS-II Nonverbal Cluster Composite Cluster	89	0.151	0522

4) Key Outcomes and Other Achievements.

We have engaged our collaborators in multiple discussions regarding study progress in Ukraine. Our major barrier has been the inability to perform research activities at the two sites that require study participants to travel or to come into the research sites in person. The prevalence of infection, low rates of vaccination in the population, and travel bans continue through the present time. Some alternatives are being considered (telephone administration of child behavior questionnaires, and to develop and test a telemedicine approach).

Additional Questions

Final Year. What do you plan to do between now and May 2022* to accomplish your CIFASD4 goals and objectives? *For those with UH2s funded in 2021, what are your goals for year two of your support?

- We have completed Aim 1b. In the next 6 months, we will perform the analyses described in Aim 1a, 1c, 2a, for the cohort retained at each age grouping (birth, infancy, preschool, and school age, and integrate biomarker data (miRNA, COR, cytokines) as available.
- We will perform the analysis of genome sequence data for the mother/child pairs with and without FASD.
- We plan a four session Ukrainian national webinar on FASD in February, 2022, that will include CIFASD presenters from the Ukraine investigators as well as Jeff Wozniak and Christie Petrenko. The goal will be to engage medical trainees, as well as a large FAS parent support group.

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

- We will provide the WGS data for Susan Smith's UH2 project, and will compare results with the CIFASD genetics investigators.
- Provided samples and data for Weinberg UH2

Administrative Supplements. For this reporting period (June 2021 to present), please list any Administrative Supplement associated with this award and a sentence or two about what it entails and its current status.

- Supplement for biomarker of exposure – we have analyzed a small set of dried blood spots for newly recruited mothers – we will perform the final analysis for the remaining maternal samples received in November, 2021.
- 3U01AA014835-15S1 Supplement for HIV/alcohol pilot data – no additional progress with halt in research data collection.

Publications/Abstracts

Publications [Accepted & In Press]

Coles CD, Kable JA, Granovska IV, Pashtepa AO, Wertelecki W, Chambers CD. Measurement of neurodevelopmental effects of prenatal alcohol exposure in Ukrainian preschool children. Child neuropsychology : a journal on normal and abnormal development in childhood and adolescence. 2021 November;27(8):1088-1103. PubMed PMID: 33982636; DOI: 10.1080/09297049.2021.1919298.

Kable JA, Coles CD, Jones KL, Yevtushok L, Kulikovsky Y, Zymak-Zakutnya N, Dubchak I, Akhmedzhanova D, Wertelecki W, Chambers CD. Infant Cardiac Orienting Responses Predict Later FASD in the Preschool Period. Alcoholism, clinical and experimental research. 2021 February;45(2):386-394. PubMed PMID: 33277942; PubMed Central PMCID: PMC7887046; DOI: 10.1111/acer.14525.

Kautz-Turnbull C, Petrenko CLM, Handley ED, Coles CD, Kable JA, Wertelecki W, Yevtushok L, Zymak-Zakutnya N, Chambers CD. Partner influence as a factor in maternal alcohol consumption and depressive symptoms, and maternal effects on infant neurodevelopmental outcomes. Alcoholism, clinical and experimental research. 2021 June;45(6):1265-1275. PubMed PMID: 33999430; PubMed Central PMCID: PMC8254755; DOI: 10.1111/acer.14612.

Bandoli G, Jones K, Wertelecki W, Yevtushok L, Zymak-Zakutnya N, Granovska I, Plotka L, Chambers C. Patterns of Prenatal Alcohol Exposure and Alcohol-Related Dysmorphic Features. Alcoholism, clinical and experimental research. 2020 October;44(10):2045-2052. PubMed PMID: 32772389; PubMed Central PMCID: PMC7722075; DOI: 10.1111/acer.14430.

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

Coles CD, Kalberg W, Kable JA, Tabachnick B, May PA, Chambers CD. Characterizing Alcohol-Related Neurodevelopmental Disorder: Prenatal Alcohol Exposure and the Spectrum of Outcomes. Alcoholism, clinical and experimental research. 2020 June;44(6):1245-1260. PubMed PMID: 32173870; DOI: 10.1111/acer.14325.

Sowell KD, Holt RR, Uriu-Adams JY, Chambers CD, Coles CD, Kable JA, Yevtushok L, Zymak-Zakutnya N, Wertelecki W, Keen CL. Altered Maternal Plasma Fatty Acid Composition by Alcohol Consumption and Smoking during Pregnancy and Associations with Fetal Alcohol Spectrum Disorders. Journal of the American College of Nutrition. 2020 March;39(3):249-260. PubMed PMID: 32240041; PubMed Central PMCID: PMC8011805; DOI: 10.1080/07315724.2020.1737984.

Bodnar TS, Raineki C, 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. Immune network dysregulation associated with child neurodevelopmental delay: modulatory role of prenatal alcohol exposure. Journal of neuroinflammation. 2020 January 28;17(1):39. PubMed PMID: 31992316; PubMed Central PMCID: PMC6988366; DOI: 10.1186/s12974-020-1717-8.

Chambers CD, Coles C, Kable J, Akshoomoff N, Xu R, Zellner JA, Honerkamp-Smith G, Manning MA, Adam MP, Jones KL. Fetal Alcohol Spectrum Disorders in a Pacific Southwest City: Maternal and Child Characteristics. Alcoholism, clinical and experimental research. 2019 December;43(12):2578-2590. PubMed PMID: 31688971; PubMed Central PMCID: PMC6904497; DOI: 10.1111/acer.14213.

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. Alcoholism, clinical and experimental research. 2019 September;43(9):1887-1897. PubMed PMID: 31329297; PubMed Central PMCID: PMC6722014; DOI: 10.1111/acer.14148.

Coles CD, Kable JA, Granovska IV, Pashtepa AO, Plotka LD, Dolhov VB, Wertelecki W, Jones KL, Chambers CD. Gestational age and socioeconomic status as mediators for the impact of prenatal alcohol exposure on development at 6 months. Birth defects research. 2019 July 15;111(12):789-796. PubMed PMID: 30378744; PubMed Central PMCID: PMC6494703; DOI: 10.1002/bdr2.1408.

Montag AC, Romero R, Jensen T, Goodblanket A, Admire A, Whitten C, Calac D, Akshoomoff N, Sanchez M, Zacarias M, Zellner JA, Del Campo M, Jones KL, Chambers CD. The Prevalence of Fetal Alcohol Spectrum Disorders in An American Indian Community. International journal of environmental research and public health. 2019 June 20;16(12). PubMed PMID: 31226736; PubMed Central PMCID: PMC6617116; DOI: 10.3390/ijerph16122179.

Xu R, Honerkamp-Smith G, Chambers CD. Statistical sensitivity analysis for the estimation of fetal alcohol spectrum disorders prevalence. Reproductive toxicology (Elmsford, N.Y.). 2019 June;86:62-67. PubMed PMID: 30959091; PubMed Central PMCID: PMC6561731; DOI: 10.1016/j.reprotox.2019.04.002.

Tseng AM, Mahnke AH, Wells AB, Salem NA, Allan AM, Roberts VH, Newman N, Walter NA, Kroenke CD, Grant KA, Akison LK, Moritz KM, Chambers CD, Miranda RC. Maternal circulating miRNAs that predict infant FASD outcomes influence placental maturation. Life science alliance. 2019 April;2(2). PubMed PMID: 30833415; PubMed Central PMCID: PMC6399548; DOI: 10.26508/lsa.201800252.

Bandoli G, Coles CD, Kable JA, Wertelecki W, Yevtushok L, Zymak-Zakutnya N, Wells A, Granovska IV, Pashtepa AO, Chambers CD. Patterns of Prenatal Alcohol Use That Predict Infant Growth and Development. Pediatrics. 2019 February;143(2). PubMed PMID: 30610099; PubMed Central PMCID: PMC6361345; DOI: 10.1542/peds.2018-2399.

Bodnar TS, Raineki C, Wertelecki W, Yevtushok L, Plotka L, Zymak-Zakutnya N, Honerkamp-Smith G, Wells A, Rolland M, Woodward TS, Coles CD, Kable JA, Chambers CD, Weinberg J. Altered maternal immune networks are associated with adverse child neurodevelopment: Impact of alcohol consumption during pregnancy. Brain, behavior, and immunity. 2018 October;73:205-215. PubMed PMID: 29738852; PubMed Central PMCID: PMC6344127; DOI: 10.1016/j.bbi.2018.05.004.

Coles CD, Kable JA, Taddeo E, Strickland D. GoFAR: improving attention, behavior and adaptive functioning in children with fetal alcohol spectrum disorders: Brief report. Developmental neurorehabilitation. 2018 July;21(5):345-349. PubMed PMID: 29313400; PubMed Central PMCID: PMC6314185; DOI: 10.1080/17518423.2018.1424263.

Sowell KD, Uriu-Adams JY, Van de Water J, Chambers CD, Coles CD, Kable JA, Yevtushok L, Zymak-Zakutnya N, Wertelecki W, Keen CL. Implications of altered maternal cytokine concentrations on infant outcomes in children with prenatal alcohol exposure. Alcohol (Fayetteville, N.Y.). 2018 May;68:49-58. PubMed PMID: 29453023; PubMed Central PMCID: PMC5820219; DOI: 10.1016/j.alcohol.2017.08.006.

May PA, Chambers CD, Kalberg WO, Zellner J, Feldman H, Buckley D, Kopald D, Hasken JM, Xu R, Honerkamp-Smith G, Taras H, Manning MA, Robinson LK, Adam MP, Abdul-Rahman O, Vaux K, Jewett T, Elliott AJ, Kable JA, Akshoomoff N, Falk D, Arroyo JA, Hereld D, Riley EP, Charness ME, Coles CD, Warren KR, Jones KL, Hoyme HE. Prevalence of Fetal Alcohol Spectrum Disorders in 4 US Communities. JAMA. 2018 February 6;319(5):474-482. PubMed PMID: 29411031; PubMed Central PMCID: PMC5839298; DOI: 10.1001/jama.2017.21896.

Chan PH, Xu R, Chambers CD. A Study of R^2 Measure under the Accelerated Failure Time Models. Communications in statistics: Simulation and computation. 2018;47(2):380-391. PubMed PMID: 29628607; PubMed Central PMCID: PMC5881951; DOI: 10.1080/03610918.2016.1177072.

Publications [In Preparation & Submitted]

Poster Abstracts and Presentations

Chambers, C.D., Coles, C.D., Kable, J.A., Wells, A., Wertelecki, W., Yevtushok, L., Zymak-Zakutnya, N., Granovska, I., Plotka, L., " Does Infant Breastfeeding Modify Cognitive Functioning in Children Prenatally Exposed to Moderate to Heavy Amounts of Alcohol?" Presented Virtually at the 2021 Research Society of Alcoholism Meeting. (June 19 - June 23, 2021)

Tseng, A.M., Balaraman, S., Allan, A.M., Chambers, C., Miranda, R.C., and the CIFASD. "Endocrine MiRNAs in Pregnant Women Predictive of FASD Infant Outcomes, Control Placental Trophoblast Growth, Survival, and Maturation." Presented at the 41st Annual Scientific Meeting of the Research Society on Alcoholism, San Diego, CA (June 2018) Published: Alcoholism: Clinical and Experimental Research 4;42(S1) (2018).

Bodnar, T.S., Raineki, C., Wertelecki, W., Yevtushok, L., Plotka, L., Zymak-Zakutnya, N., Well, A., Honerkamp-Smith, G., Coles, C.D., Kable, J.A., Chambers, C.D., Weinberg, J., the CIFASD. "Cytokine Disturbances Associated with Prenatal Alcohol Exposure in Children: Implications for Health and Development." Presented at the 41st Annual Scientific Meeting of the Research Society on Alcoholism. San Diego, CA (June 2018) Published: Alcoholism: Clinical and Experimental Research 42(S1):46A (2018).

Coles, C.D., Kable, J.A., Mesa, D.A., Coleman, T.P., Jones, K.L., Yevtushok, L., Kulikovsky, Y., Wertelecki, W., Chambers, C.D. "Early Identification of Effects of Prenatal Alcohol Exposure: Infant Cardiac Orienting Response

as a Biomarker." Presented at the 41st Annual Scientific Meeting of the Research Society on Alcoholism, San Diego, CA (June 2018) Published: Alcoholism: Clinical and Experimental Research 42(S1) (2018).

Chambers, C.D., Wells, A., Xu, R., Wertelecki, W., Coles, C.D., Kable, J., Zymak-Zakutnya, N., Yevtushok, L. A Growth Modeling Approach to Predicting Future Neurodevelopmental Performances in Infants with Prenatal Alcohol Exposure. Presented at the 41st Annual Scientific Meeting of the Research Society on Alcoholism, San Diego, CA (June 2018) Published: Alcoholism: Clinical and Experimental Research 42(S1):247A (2018).

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., and the CIFASD. "Alcohol Intake and Immune Function: Associations between Maternal Immune Networks and Child Neurodevelopmental Outcome." Presented at the 41st Annual Scientific Meeting of the Research Society on Alcoholism, San Diego, CA (June 2018) Published: Alcoholism: Clinical and Experimental Research 42(S1):247A (2018).

Principal Investigators: Claire Coles/Subaward PI Therese Grant
Institutions: Emory University School of Medicine/ University of Washington
CIFASD4 Project Title: Fetal Alcohol Spectrum Disorder in Adults: Health and Neurobehavior
Grant Number: U01 AA026108

Specific Aims

Specific Aim 1: *Establish a registry of individuals with prenatal alcohol exposure (PAE) who are willing to participate in future research, beginning with those who are enrolled in the large Seattle and Atlanta studies and survey their health status.*
Specific Aim 2*: In a subsample of adults selected from these two cohorts (N=240, 120 per site), evaluate in depth current status in areas supporting adult physical wellbeing and social functioning,* including dysmorphology, mental health, substance use and aspects of adaptive and social functioning where deficits may not have been apparent earlier in life.
Specific Aim 3: *This aim 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 behavior measures collected in this project.*

Accomplishments

1) Major Activities.

Major Activities. In the lasts 6 months, as noted previously, at both Atlanta and Seattle sites, our major focus has been on recruitment and data collection within the context of the significant impediments associated with the COVID-19 pandemic. In addition, of course, we wrote the application for CIFASD 5 and began data analysis and preparation of papers and abstracts.

Data Collection for Registry, Tier 1 and Tier 2. During the period from June 2021 to November 2021, both sites continued with data collection including both the original protocol and that associated with the COVID supplement. Due to the continued COVID pandemic, we adhered to modified protocols required by the Universities and by safety considerations and approved by the IRBs at both Universities. Whenever possible, remote data collection procedures are in place. There have been no problems implementing these protocols for Tier 1 activities. For Tier 2, we can only administer questionnaires remotely and "in person" activities continue for many aspects. However, we are allowed only one individual at a time for testing due to COVID safety precautions. As a result, some participants have completed only part of Tier 2; we are bringing them in when available during the time frame in which we are allowed to have participants at clinical sites. Usually, we have combined these activities with those required for one or both of the Supplements (COVID and Diversity) that were received for this award. As of November 1, 2021, Seattle has completed 204 Tier 1 and 94 Tier 2 evaluations; Atlanta has completed 131 Tier 1 and 115 Tier 2 evaluations. For Tier 1, data collection is currently at 67% of goal; For Tier 2, it is at 91%. At both sites 169 COVID protocols have been completed (Atlanta:73; Seattle: 96). Presently, funds for staff salaries are nearly exhausted due to the delays associated with COVID; however, both sites are committed to continuing to collect data as long as funds are available.

2) Specific Objectives

Specific Objectives.

- Establish a Registry: This has been done at both sites and data combined in REDCap and recruitment into the registry is done as participants are identified and recruited. Currently, 296 individuals have agreed to participate in the Registry.
- Tier 1- Heath and Demographic survey. As of the end of October 2021, 336 subjects have completed these forms.
- Tier 2: In Depth Testing. Selected individuals were recruited and seen for testing of current status. As of the end of October, 2021, 219 individuals at both sites had completed assessment (91% of total goal).
- Use of data collection and storage instruments using REDCap. We continue to use this mechanism for data entry and for transfer of information to the Informatics Core in Indiana.
- Blood samples are being collected and retained in cold storage until they can be sent to Vancouver for analysis.

- 2-D photographs were taken and sent to Dr. Suttie without problems. Dr. Suttie has reviewed the 2-D photographs and concluded that they are not useful for his purposes. We are continuing to collect these and will retain them.
- Saliva samples have been collected in Atlanta and Seattle and sent to Indiana for genetics studies.
- Telemedicine for dysmorphology examinations for FASD in Atlanta. With COVID restrictions, we changed the plan for dysmorphology assessment. Dr. Gaby Ritfeld carried out dysmorphology evaluations until leaving for a new position in Arizona in August, 2021. In September/October, 2021, Zvi Shapiro, PhD, was trained to carry out this function by the Dysmorphology core. Previous participants will be assessed when possible.

3) Significant Results.

All results are preliminary as data collection is not complete.

Participant Characteristics: Of the 336 participants identified to date at both sites, 41.5% are Male and 58.5% are Female; 8% are American Indian, 38.5% are African-American, 43.7% are Caucasian and 9.5% report being biracial; 93.2% are Non-Hispanic. 44.4% percent have never married (with significantly fewer individuals with FAS married), while 41.6% are married or living with a partner, 2.8% separated and 10.5% divorced. 66.4% have biological children. The majority report completing high school (23.8%) or attending college or trade school (54.6%) while 21% did not graduate from high school; 53,4% percent report working full time and 22.6%, part time while 12.3% are disabled and 11.1% unemployed.

Health Status: Analysis of the Health Questionnaire reveals significant differences in a number of health problems for alcohol affected individuals. Prenatal alcohol exposure was associated with greater self-report of problems with: Vision, Hearing, Dental, Gastrointestinal, Incontinence, Prediabetes, Thyroid issues, Allergies Arthritis, Seizures, ADHD and Cancer. In addition, those with PAE reported much higher lifetime incidence of mental health disorders, particularly depressive symptoms, and diagnosis of depression ($X^2_{(4)}$ =10.98, p<.03) and anxiety ($X^2_{(6)}$ =22.86, p<.001), than did those in the contrast groups.

Neurocognition. The Fluid Cognition measures from the NIH Toolbox were used to measure cognition, anticipating accelerated aging effects in executive functioning, attention and memory. The Flanker Task, a measure of executive functioning, was most sensitive to alcohol effects ($F_{(1,113)}$=17.93, p<.000); members of the PAE group performed significantly less well on all subtests and on the overall Cognitive Composite ($F_{(1,113)}$=22.7, p<.000), Cognitive outcomes correlated with measures of vascular pathology (r= -.225), suggesting a possible mechanism for cognitive impairment as well as raising concerns about future cognitive decline as vascular pathology may increase with age.

Substance Use. Two hundred and four participants completed the Tier 2 Drug Checklist and reported a significant level of use of various substances although there were no significant difference in reported use by alcohol group. Males in the Control group reported higher levels of alcohol use than did those in the Alcohol Groups. Lab results confirmed self-report but indicated a significantly higher level of out of range GGTP results for those in the alcohol group ($X^2_{(1)}$=3.79, p=.05).

4) Key Outcomes and Other Achievements.

Key Outcomes and Other Achievements. Analysis of the Health Questionnaire results reveals significant differences in a number of health aspects for alcohol affected individuals. In addition this group reports greater childhood adversity and lower SES and these factors are also related to outcome. Results were presented at the Proof On-Line Meeting in September 2020 and at the Fetal Alcohol Study Group and Research Society on Alcoholism Annual Meeting in June, 2021.

Additional Questions

Final Year. Several months into Year 5, we are still coping with the limitations imposed by the pandemic. Year 5 was envisioned to be a year of data analysis and report writing with lower staff and subject costs. Due to the delays occasioned by COVID, we are continuing to identify participants and collect as much data as possible with the understanding that we will soon exhaust existing funds. We will begin data analysis as rapidly as possible when data collection is no longer possible. Our data management via REDCap has made assessing data much easier than previously which will facilitate these efforts. We are encouraged, however, by our preliminary data analyses that suggest strong effects that will be identifiable even if we are not able to reach data collection goals.

CIFASD4 Synergy

Cross-Site Collaboration. A major focus throughout the study has been collaboration not only with our subcontractors in Seattle but with Dr. Weinberg's project in Vancouver maintained through frequent consultations and Zoom conferences. We also continue to work with Drs Jones/ and Dr. Foroud's projects and have collected photographs for Dr. Suttie.

Collaboration with Associated Studies. In the current grant year, we continued to collaborate with two other investigators who have "associated" studies. In Atlanta, for her R21 protocol, now in a no-cost extension, Julie A Kable, PhD collected data on micro vascularization and cardiac health and frontal lobe functioning in 80 adults participating in Tier 2. This collaboration has already resulted in a publication and a number of abstracts. 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."

Administrative Supplements. We were fortunate enough to receive two Administrative Supplement for this award.

1. COVID Supplement (Atlanta and Seattle)

There were several specific aims of this award.

1. Determine impact of the COVID-19 pandemic on physical and mental health (including substance use) of those with PAE and SES-matched controls
2. In the Atlanta Cohort only, determine the relative rate of exposure to the COVID 19 virus by using antibody testing.
3. Determine the mortality rates, particularly during the peak periods of the COVID-19 pandemic, of two original cohorts (n=1000) and control for gender and lifestyle factors that may influence early mortality using the National Death Index (NDI)records.

Accomplishments

Results of COVID Questionnaire. To date 169 participants (108 PAE, 61 controls) completed the COVID protocol with 7.3% of the Exposed group and 2.4% of FAS/DYS group having COVID vs 8.2 of Controls ($X^2_{(4)}$=8.61, p=.07). More individuals with FAS (31.7%) reported being vaccinated than controls (14.8%). Those in the control group reported more serious illness (X^2=12.33 p<.01) and death (X^2=8.5, p-.075) in their immediate families. This group also reported more problems in caring for older family members and those with disabilities than did those in the alcohol group (X^2=37.69 p<.001) perhaps because they are more likely to have such responsibilities. Those in the FAS group were less likely to report a negative impact on their ability to exercise than the other groups (X^2=18.67, p<.04) and there was a similar trend for sleep problems (X^2=16.22, p=.09). All groups reported high levels of anxiety and depressed mood.

Mortality. Application was made to the Centers for Disease Control and Prevention for access to the National Death Index (NDI) and both Universities provided IRB approval for this study. Data were extracted from longitudinal data sets and provided to the NDI. Results for Years 2010 to 2020 were received November 29, 2021 but have not yet been cleaned and validated.

2. Diversity Supplement (3U01AA026108-04S1). Gaby Ritfeld, MD, PhD, received a Diversity Supplement entitled, *Neurobehavioral and Physical Health Outcomes in Offspring of Individuals with Fetal Alcohol Spectrum Disorders.*

This research project has three aims:

1. Assess the neurobehavioral and physical health status of the offspring of adults with FASD, including prevalence of psychiatric problems, aspects of neurocognitive and adaptive functioning, and prevalence of FASD
2. Identify parental determinants of health and disease in children of individuals with FASD
3. Determine the impact of environmental factors, including adverse childhood experiences (ACES), on the health outcomes in offspring of FASD-affected individuals.

Although Dr. Ritfeld has moved to Arizona, data collection continues in the Atlanta Lab and remotely from Arizona. To date 34 dyads have been assessed.

Preliminary Results. By July, 2021, 26 parent child dyads had been tested with an average Child age of 12 years. Offspring of individuals with FASD displayed below normal neurocognitive function but this was not different from controls and can be attributed to SES. No significant behavior problems were reported in either group. Children of individuals with FASD were less likely to be receiving academic support than controls despite equal levels of problems in this area.

Publications/Abstracts

Publications [Published; Accepted & In Press]

Kable, JA**,** Mehta, PK, & Coles, CD. (2021) Alterations in insulin levels in adults with prenatal alcohol exposure. Alcoholism Clinical and Experimental Research, 45 (3), 500-506. https://doi.org/10.1111/acer.14559. [PMID: 33486796

Ritfeld,G, Kable,JA, & Coles,CD. *Socioeconomic wellbeing/impairment in a cohort of individuals prenatally exposed to alcohol: exploring parenting styles as a determinant of outcome.* JAACAP. 2020;59(10): S215.

Publications (In preparation and submitted)

Coles, CD, Grant, T,. Kable, JA, Stoner, S, Perez, A, and the CIFASD (2021, in review) Prenatal Alcohol Exposure and Mental Health at Midlife: A Preliminary Report on Two Longitudinal Cohorts. Submitted to Alcoholism: Clinical and Experimental Research.

Poster Abstracts and Presentations

Coles. CD (2020) *Exploring an undiscovered country: Effects of prenatal alcohol exposure in adulthood.* Presented at the PROOF Alliance Annual Meeting, October 22, 2020.

Coles, CD, Grant, T, Weinberg, J, Kable, JA, Radin, S., Smith-Steward, T, Perez, A and the CIFASD (2021) *Alcohol Affected Adults' Self-Reported Health and Mental Health Status: Preliminary evidence of long-term effects from a cross site study.* Presented at a Symposium at the Research Society on Alcoholism Annual Meeting, June, 2021.

Kable, JA,& Mehta, PK, Coles, CD (2021) *Prenatal alcohol exposure and cardiovascular risk: Preliminary results from a prospective cohort of adults first identified while in utero.* Presented at a Symposium at the Research Society on Alcoholism Annual Meeting, June, 2021. Presented at a Symposium at the Research Society on Alcoholism Annual Meeting, June, 2021.

Coles, CD, Grant, T, Kable, JA, Rashid, F, and Mehta, PK (2021) *Prenatal alcohol exposure and cognitive outcomes in midlife: Long-term effects in two longitudinal cohorts*. Poster presented at the Research Society on Alcoholism Annual Meeting, June, 2021.

Ritfeld G, Kable JA, Coles CD. *Socioeconomic wellbeing/impairment in a cohort of individuals prenatally exposed to alcohol: exploring parenting styles as a determinant of outcome*. American Academy of Child and Adolescent Psychiatry 67th Annual Meeting.

Ritfeld, G (July, 2021) *Preliminary analysis of neurobehavioral and physical health outcomes in offspring of individuals with Fetal Alcohol Spectrum Disorders*. Presented at NIH Workshop for Diversity Supplement recipients.

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: U01 AA026103

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, 609 individuals have enrolled, primarily from the United States and Canada, with 29 participants enrolling since June 1, 2021. Slightly more than half of all participants report themselves as Caucasian (54.4%). There is much diversity in the remaining individuals who reported a race, including Native American or First Nation (5.74%), African (7.39%) or more than one race (12.3%; 20.2% unknown race). The sample is 42.8% female, 41.2% male, and 16% unknown. The study includes a consent for adult participants or parents of minors, as well as an assent. As a result, participants include individuals aged 1 - 77 years, with 66.7% under 18 years (minor). Following consent, online forms gather information on alcohol exposure: 64% of participants have a biological mother with behavioral indicators of drinking during pregnancy and the majority of the remaining 36% have reported either at least minimal PAE or an FASD. 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 346 individuals and saliva from 287 individuals.

We have made progress in several areas which will have significant impact over the remainder of the grant period. We relaunched our recruitment efforts, which we kicked off by sending letters to over 200 patients from Dr. David Weaver, a clinician in our department who had an FAS clinic several years ago. We re-introduced our presence on social media by creating a DiG FASD Instagram account and then began posting a “meet the researchers” and a “study Q & A” series across all our social media platforms (Instagram,

Twitter, and Facebook). These posts mirror updates made to the study website: a page to learn more about the researchers, a page with commonly asked questions and answers, a place to watch study videos, view and download study flyers, and more. We also started reaching out to FASD-related organizations to request help promoting DiG FASD. We are organizing with FASD United and Tom Donaldson to include information about our study in their weekly newsletter starting December 6, to push an email to individuals in their database, and to join their January, 2021 monthly conference call with their FASD United affiliates. Since June 1, 2021, 18 individuals have uploaded a photograph and 14 individuals have provided saliva. Of the 90 participants enrolled since last year's report, 13 enrolled within the last month, since we began this outreach. This is equivalent to almost a 200% increase in enrollments compared to the prior 12 months.

In combination with our recruitment efforts, we have also participated in FASD advocacy events, such as Run FASD. This was a virtual event hosted by FASD United to raise awareness for FASD in September. We will also be interviewed by the FASD Hope podcast for an episode that will air in early 2022.

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. We integrated the online Brief Assessment of Individual Neurobehavior (BRAIN) assessment created by Dr. Mattson into our study. Since June 1, 2021, we have had our first two DiG FASD participants complete the BRAIN-online. 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. This questionnaire was launched during this reporting period, and we have had 5 participants complete it. 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.

DiGFASD participants consent to broad sharing of their data and to being contacted for other studies for which they are eligible. This registry, with the depth of phenotypic information as well as genetic data, is poised to be extremely beneficial to CIFASD5 investigators. Therefore, we focused our efforts on identifying individuals with prenatal alcohol exposure (PAE) who would be informative for WES and genome-wide association study (GWAS) analyses from previous CIFASD 2 and CIFASD 3 phases. We will continue to perform WES on appropriate DiGFASD and CIFASD4 individuals with PAE, in addition to obtaining complementary GWAS data for these samples. This effort will result in an estimated 650 additional individuals with GWAS and 220 with PAE and WES. The combined GWAS dataset (pre-QC) is projected to have 1,196 individuals with GWAS data, and 713 individuals with PAE and WES data.

In addition to analyses using Dr. Suttie's facial signature phenotypes from 2D and 3D images, we performed analyses in the WES sample and the updated GWAS sample from CIFASD2 and CIFASD3 using four spatial working memory measures. As requested by Dr. Torii, we examined results in the *APOE* gene, due to the correlation of *APOE* expression and spatial motor learning task results in mice. There was modest evidence (p=0.02) that *APOE* expression was associated with the delayed matching to sample z-score. This z-score is a composite of correct responses for the task of choosing the original pattern after seeing that pattern followed by 4 different patterns. Individuals in the CIFASD WES sample with PAE, who had a variant in *APOE,* had lower z-scores than those without a variant. GWAS results for single nucleotide polymorphisms (SNPs) indicated a genotype by PAE association with SNPs in a region upstream of the *APOE* gene with the same z-scores in individuals of African ancestry (AA), including a functional transcript variant rs584007 (p=0.0081). When examining individuals with the minor allele A (minor allele frequency = 0.21 in the AA sample), those with PAE had lower Z-scores compared to individuals without PAE. We continue to collaborate with Dr. Torii, her graduate student Amy Hwang, and Sarah Mattson.

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.

- A data dictionary is available for all CIFASD projects
- Most CIFASD investigators submit data quarterly, as applicable
    - Adult Study (Coles/Grant) N=356
    - Biomarkers (Torii) N=75 animals, fastq files; N=40 blood samples (clinical)
    - Decision tree (Mattson) N=328
    - Demographics (Mattson) N=222
    - Human Genetics (Foroud) N=460
    - Imaging (Suttie) N=253 Ultrasound images, 3d landmark data for N=597
    - Immune (Weinberg) N=72
    - Intervention (Petrenko) N=105
    - Microbiome (Blanchard, Mooney) N=38 animals
    - Mouse Genetics (Parnell) N=63 genes for 12 mice (KO + WT)
    - Neurobehavior (Mattson/Wozniak) N=262
    - Neuroimaging (Wozniak) N=156
    - Ukraine (Chambers) N=167 mothers and N=141 infants (N=308 total); 118 ultrasound images, 34 3D images
- All data are available to all CIFASD investigators to be shared across components

Key Outcomes and Other Achievements.

- In addition to a standard operating procedure (SOP) in place to handle requests for archived CIFASD data, we have created am SOP to dictate how data are shared with the approved investigator, and are finalizing an SOP to describe how the one-year annual report is presented to the Data Access Committee. Since June, 2021, one request by Sarah Oh, was re-submitted and approved.

Additional Questions

Final Year. Aims 1-2: We will send final samples for WES and GWAS, clean and combine the data, and perform final analyses implementing the 2d and 3d facial signatures from Dr. Suttie, the neurocognitive variables ran previously, and if sample size permits, BRAIN measures, and any health-related phenotypes recommended by Dr. Coles or Dr. Weinberg. We will document all cleaning and genetic files for archived use and sharing. **Aim 3:** SOPs for sharing data, one-year follow-up, and data-closeout will be finalized. A sentence describing available archived data, and the corresponding data sharing link will be crafted and approved by the DAC for use in CIFASD slides and manuscripts, both for CIFASD 2-3 data and (separately) for CIFASD4 data. All CIFASD4 data will be evaluated and updated to prepare for archiving. The DAC will work to create and approve a link on the CIFASD.org website for describing CIFASD4 data for sharing.

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 continue to collect saliva for the DiG-FASD online project*.

- Four 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 analyses.
- As the DiG-FASD project incorporates subsets of CIFASD4 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.
- Mike Suttie (*Facial Imaging*) has developed a facial risk score from the 2D photos. The scores are part of the quarterly upload of data to the Central Repository. We analyzed

these scores in our WES samples however the small sample size and non-replication of results based on scores from 3D images preclude interpreting results based on the 2D scores.

C. *We implemented an online neurocognitive assessment (Mattson).*

- Once the number of people completing the BRAIN is adequate, 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 as they become available.

D. *Outreach/dissemination*:

- Tom Donaldson and Kathy Mitchell continue to promote our DiG-FASD study in their talks and in meetings by showing the promotion slide.
- FASD United has created several images to help advertise the DiG-FASD study on Facebook and other social media outlets.
- FASD United will be highlighting the study with a feature story in an upcoming weekly roundup newsletter.
- FASD United will send out emails to families and individuals on their list serve to promote the study.
- FASD United will be introducing us to their affiliate network to encourage more organization to help DiG FASD recruit participants.
- We continue to work with Dr. Petrenko to recruit and enroll participants for DiG FASD. We have also shared materials on our social media pages, to aid Dr. Petrenko in recruitment for focus groups for her mobile app.

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

- Genes identified from the animal model components are examined 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 met with Dr. Susan Smith on November 18 to discuss a strategy for her analyses of GWAS data. We will run the GWAS in parallel on neuropsych variables suggested by Jeff Wozniak and compare results. This will ensure analyses are performed properly.

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: Sarah Mattson
Institution: San Diego State University
CIFASD4 Project Title: Multisite Neurobehavioral Assessment of FASD
Grant Number: U01 AA014834

Specific Aims [from original funding application]

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, FASD-Tree (formerly the decision tree or the eTree), validation test battery, and the BRAIN-online (formerly FONS). Activities and achievements are described in the following sections and reflect only work completed in this reporting period (June's 2021 to present).

1) Major Activities. In the final year of this project, we have focused on analyzing data and writing papers. We also submitted the renewal application in August of 2021. Since June of 2021, we have completed 4 papers: 1 has been accepted, 1 is submitted, and 2 will be submitted in the next month. They are described below. We have also continued to collect data, although we remained slowed due to the COVID-19 pandemic. We are about to invite research subjects back into the lab; this should happen in the next couple of weeks.

Data Collection:
Due to the COVID-19 pandemic, all in-person testing was paused in March of 2020. We are about to re-initiate in-person testing (early December 2021). We have continued to collect data using BRAIN-online. We are very close or above our project goals for the 5-year period. Our current sample sizes are listed in Table 2.

Table 2. Number of Subjects Tested During CIFASD4

CIFASD Site	FASD-Tree	NP Testing	BRAIN-online
San Diego	234	114	103
Minneapolis	92	101	13
Total	332	215	116
5y Goal	350	210	100
% of 5y Goal	95%	102%	116%

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.
Papers completed:
1. **Risk Score Paper (in press, November 2021).** The purpose of this study was to to develop an efficient and easily calculable risk score that can be used to identify an individual's risk of having been exposed to alcohol prenatally. Subjects from CIFASD-2 (n=325, the development cohort, DC) and CIFASD-3 (n=523, the comparative cohort, CC) were included. The risk score was accurate in both cohorts. Subjects were accurately classified in the DC ($\chi 2 = 78.61$, $p < .001$) and CC ($\chi 2 = 86.63$, $p < .001$). We also tested risk groups based on the risk score (low vs. high risk) and this model also performed well in the DC (ROC = .835 [SE = 0.024, $p < .001$])

and CC (ROC = .786 [SE = .021, p <.001]. In the AE-CC and CON-CC, modest but significant associations between the risk score and executive function (AE-CC: r = -.20, p = .034; CON-CC: r = -.28, p <.001) and IQ (AE-CC: r = -.20, p = .034; CON-CC: r = -.28, p <.001) were found. In sum, our risk score was successful in distinguishing alcohol-exposed subjects from controls and has significant clinical potential.

2. **FASD-Tree Paper (submitted, November 2021).** In this study, we combined the decision tree (Goh et al., 2016) with the risk score (Paper 1 above) into the FASD-Tree and tested its accuracy and validity in a new sample. 312 subjects were included from CIFASD-4 (San Diego and Minneapolis). The FASD-Tree was successful in discriminating youth with histories of prenatal alcohol exposure from those with no such exposure indicating its validity as an FASD screening tool. Overall accuracy rates for FASD-Tree components ranged from 76.5%-84.2% and both the decision tree outcome and risk score, as well as their combination, resulted in fair to good discrimination (AUC = .758-.819) of youth with histories of prenatal alcohol exposure. While most patients were correctly classified, those who were misclassified differed on IQ and ADHD symptoms. Race, ethnicity, and sex did not affect results. Thus, the FASD-Tree is an accurate and valid screening tool for FASD.

Analysis completed:

3. **NP-Tree Paper (in preparation, to be submitted December, 2021).** The purpose of this study was to examine neuropsychological performance related to the outcome of the FASD-Tree. We compared neuropsychological test results for subjects that had also completed the FASD-Tree. 175 subjects were included from CIFASD-4 (San Diego and Minneapolis). Overall accuracy of the FASD-Tree was 82%. Subjects who were classified as proposed AE by the FASD-Tree were more likely to have a lower FSIQ (OR=0.93, 95% CI: 0.91-0.95, p<0.001), perform worse on measures of executive function (OR=0.22, 95% CI: 0.12-0.40, p<0.001), to be rated as having more behaviors associated with executive dysfunction (OR=1.16, 95% CI: 1.11-1.21, p<0.001), externalizing problems (OR=1.10, 95% CI: 1.07-1.13, p<0.001), internalizing problems (OR=1.06, 95% CI: 1.03-1.10, p<0.001), having more behavioral symptoms on the BSI (OR=1.12, 95% CI: 1.08-1.16, p<0.001) and fewer adaptive skills (OR=0.85, 95% CI: 0.80-0.89, p<0.001), and to perform worse on measures of math problem solving (OR=0.93, 95% CI: 0.91-0.96, p<0.001) and numerical operations (OR=0.94, 95% CI: 0.92-0.96, p<0.001). No differences were observed on word reading (OR=1.00, 95% CI: 1.00-1.00, p>0.05). Similar relationships were found in all measures when comparing only subjects who were correctly classified by the FASD-Tree, and in all measures but internalizing problems (OR=1.03, 95% CI: 0.99 -1.01, p>0.05) when comparing only subjects with prenatal alcohol exposure. These results lend evidence in support of the FASD-Tree as an effective screening tool in clinical settings, providing an efficient and accurate way to identify patients in need of additional evaluation.

4. **BASC Validation Paper (in preparation, to be submitted December, 2021).** The purpose of this study was to compare the result of 3 commonly used parent-report measures. We traditionally use the CBCL and VABS-3 and wanted to know if we could instead use a single measure, the BASC-3. 256 subjects were included from CIFASD-4 (San Diego and Minneapolis). Across groups, significant correlations were found for test scores of adaptive (r = .86, p < .001), internalizing (r = .76, p < .001), and externalizing (r = .87, p < .001) behavior. Similar correlations were found within groups. BASC-3 sensitivity rates were 46.6%, 80.4%, and 77.9% for internalizing, externalizing, and adaptive behavior, respectively. BASC-3 specificity rates were 81.5%, 80.4%, and 79.3% for internalizing, externalizing, and adaptive behavior, respectively. BASC-3 positive predictive values were 81.7%, 87.9%, and 87.0% for internalizing, externalizing, and adaptive behavior, respectively. BASC-3 negative predictive values were 46.3%, 69.8%, and 67.0% for internalizing, externalizing, and adaptive behavior, respectively. These findings replicate previous reports of behavioral and adaptive difficulties in youth with prenatal alcohol exposure and provide support for using the BASC-3 in this population.

4) Key Outcomes and Other Achievements. We are proud of our accomplishments. The suite of 3 papers (#s1-3 above) provide important validation of our tools. We combined our previously described decision tree with the risk score into the FASD-Tree and demonstrated its utility as an FASD screening tool. We also described the relationship between the FASD-Tree and neuropsychological outcomes, further strengthening the clinical utility of our tools. Our BRAIN-online tool is newer and still being tested, but we hope to be able to present data at the 2022 RSA conference and the reverse site visit in early 2022. We also deployed BRAIN-online in a sample of young adults. Our aim was to examine normative performance on this novel tool so that it can be used in adult populations by other investigators. For this study, we have tested SDSU students between 18-25. We have other more preliminary studies in the works, including testing: the FASD-Tree as a screen for ARND, the role of IQ in behavior, co-occurring maternal exposure and neuropsychological performance, the BASC as a substitute for the CBCL and VABS in the FASD-Tree (a follow up to paper #4), parent reports of executive functioning vs. laboratory measures, and risk/resilience factors.

Additional Questions

Final Year. We have completed our data collection goals for the funding period. However, we plan to continue collecting data, both online and in person throughout the remainder of the grant. We are poised to re-invite research subjects back into the lab and this will allow us to increase the sample size of subjects with both in person testing and BRAIN-online. We plan to collect data through our collaborator in developmental behavioral pediatrics which will provide us with important comparison data. We will also continue to analyze data and write papers. We have several papers that are in various stages of development and hope to submit several of them before the end of the funding period. We will continue to work with our collaborators (Primarily Drs. Wozniak, Suttie, Jones, and Del Campo). We are making improvements to the FASD-Tree in anticipation of continued funding.

CIFASD4 Synergy. This project involves direct collaborations with several other U01 projects, including those directed by Drs. Foroud, Weatherill, 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.

Administrative Supplements. This award has one administrative supplement: 3U01AA014834-16S1. This is a "Research Supplement to Promote Diversity in Health-Related Research" and supports Carissa Zambrano. Ms. Zambrano has been supported in various training activities including coursework at SDSU. She participated in the preparation of a book chapter that was recently submitted.

Publications/Abstracts (please list from most recent first back to June/July 2017)

Publications [Accepted & In Press]

A complete list of published work can be found at: http://www.ncbi.nlm.nih.gov/sites/myncbi/sarah.mattsonweller.1/bibliography/40832384/public/?sort=date&direction=ascending

Peer-Reviewed Papers

1. Bernes, G.A., Courchesne, N.S, Hyland, M.T., Villodas, M.T., Coles, C.D., Kable, J.A., May, P.A., Kalberg, W.O., Sowell, E.R., Wozniak, J.R., Jones, K.L., Riley, E.P., **Mattson**, S.N. and the CIFASD. (2021). Development and validation of a postnatal risk score that identifies children with prenatal alcohol exposure. Alcoholism: Clinical and Experimental Research, in press. Available online 21 November 2021. doi: 10.1111/acer.14749. PMCID: In Progress (PMID: 34806190). https://pubmed.ncbi.nlm.nih.gov/34806190/

2. Bernes, G.A., Villodas, M., 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 (2021). Validity and reliability of executive function measures in children with heavy prenatal alcohol exposure: Correspondence between multiple raters and laboratory measures. Alcoholism: Clinical and Experimental Research, 45 (3), 596-607. Available online 15 February 2021. doi: 10.1111/acer.14547. PMCID: PMC7969422. https://pubmed.ncbi.nlm.nih.gov/33433001/

3. 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. (2021). Social Behaviors and Gray Matter Volumes of Brain Areas Supporting Social Cognition in Children and Adolescents with Prenatal Alcohol Exposure. Brain Research, in press. Available online 20 February 2021. doi: 10.1016/j.brainres.2021.147388. PMCID: In Process (PMID: 33621483). https://pubmed.ncbi.nlm.nih.gov/33621483/

4. Rockhold, M.N., Krueger, A.M., de Water, E., Lindgren, C.W., Sandness, K.E., Eckerle, J.K., Schumacher, M.J., Fink, B.A., Boys, C.J., Carlson, S.M., Fuglestad, A.J., **Mattson**, S.N., Jones, K.L.; Riley, E.P., Wozniak, J.R. (2021). Executive and Social Functioning Across Development in Children and Adolescents with Prenatal Alcohol Exposure. Alcoholism: Clinical and Experimental Research, 45 (2), 457-469. Available online 21 December 2020. doi: 10.1111/acer.14538. PMCID: PMC7887051. https://pubmed.ncbi.nlm.nih.gov/33349933/

5. Moore, E.M., Glass, L., Infante, M.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, 45 (1), 215-223. Available online 15 November 2020. doi: 10.1111/acer.14506. PMCID: PMC7875477. https://onlinelibrary.wiley.com/share/author/QVFBNFTXP39KNKXME3WS?target=10.1111/acer.14506

6. 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, and Wozniak, J.R. (2020). Hippocampal subfield abnormalities and memory functioning in children with fetal alcohol spectrum disorders. Neurotoxicology and Teratology, 83, 106944. Available online 21 Nov 2020.doi: 10.1016/j.ntt.2020.106944. PMCID: PMC7855420. https://pubmed.ncbi.nlm.nih.gov/33232797/

7. 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). Para-limbic structural abnormalities are associated with internalizing symptomology in children with prenatal alcohol exposure. Alcoholism: Clinical & Experimental Research, 44 (8): 1598-1608. Available online 11 June 2020doi: 10.1111/acer.14390. PMCID: PMC7484415. https://pubmed.ncbi.nlm.nih.gov/32524616/

8. Uban, K.A., Kan, E., Wozniak, J., **Mattson**, S., Coles, C.D., Sowell, E.R., & the CIFASD (2020). The relationship between socioeconomic status and brain development is attenuated in children and adolescents with prenatal alcohol exposure. Frontiers in Human Neuroscience, 14:85. Available online 08 April 2020. doi: 10.3389/fnhum.2020.00085. PMCID: PMC7156853. https://pubmed.ncbi.nlm.nih.gov/32322193/

9. 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)
https://www.ncbi.nlm.nih.gov/pubmed/32217203

10. 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
https://www.ncbi.nlm.nih.gov/pubmed/31914808

11. 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
https://www.ncbi.nlm.nih.gov/pubmed/30908651

12. **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
https://www.ncbi.nlm.nih.gov/pubmed/30964197
This paper was the most downloaded article from the journal in 2019

13. 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
https://www.ncbi.nlm.nih.gov/pubmed/30719847

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

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

16. 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
https://www.ncbi.nlm.nih.gov/pubmed/28270013

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

18. 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
https://www.ncbi.nlm.nih.gov/pubmed/30322415

19. 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
https://www.ncbi.nlm.nih.gov/pubmed/28340498

20. 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
https://www.ncbi.nlm.nih.gov/pubmed/28965651

Book Chapters

1. Hyland*, M.T., Courchesne*, N.S., Sobolewski*, C.M., Zambrano*, C., and **Mattson**, S.N. (2021, submitted). Fetal Alcohol Spectrum Disorders: Neuropsychological outcomes across the lifespan. In O. Rahman, C. Petrenko (Eds.), Fetal Alcohol Spectrum Disorders: A Multidisciplinary Approach. Springer.

2. Max, J.E., **Mattson**, S.N., Vaucher, Y.E., Nichols, S., and Nespeca, M.P. (2021, submitted). Psychiatric aspects of child neurology. In B.J. Sadock, V.A. Sadock, P. Ruiz (Eds.), Kaplan & Sadock's Comprehensive Textbook of Psychiatry, 11th Edition Philadelphia, PA: Lippincott Williams & Wilkins.
3. 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.
4. **Mattson,** S.N., Poth*, L.D., Glass*, L. (2021). Fetal Alcohol Spectrum Disorders and Other Teratogenic Conditions. In Glidden, L.M., Abbeduto, L., McIntyre, L.L, Tassé, M.J. (eds). APA Handbook of Intellectual and Developmental Disabilities, Vol. 1 (pp. 295-322). Washington, DC: American Psychological Association.
5. 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

Mattson, S.N., Jones, K.L., Chockalingam, G., Wozniak, J.R., Hyland, M.T., Courchesne, N.S., Del Campo, M., Riley, E.P., & the CIFASD. (2021). Validation of the FASD-Tree as a screening tool for fetal alcohol spectrum disorders.

Poth, L.D., Love, T., Mattson, S.N. (submitted 2021). Profiles of language and communication abilities in adolescents with fetal alcohol spectrum disorders.

In Preparation

Bernes, G.A., et al., Accuracy of an automated decision tree tool for detecting ARND.

Sobolewski, C.M., et al., Adaptive, Externalizing, and Internalizing Behavior of Children with Prenatal Alcohol Exposure: A Comparison of Three Parent-Report Questionnaires.

Hyland, M.T., et al., Correlation Between Results of an Automated Decision Tree Tool for Detecting FASD and Neuropsychological Outcomes.

Poster Abstracts and Presentations

1. Hyland, M.T., Courchesne, N.S., Bernes, G.A., Krueger, A.M., Rockhold, M.N., Wozniak, J.R., Jones, K.L., Del Campo, M., Boys, C.J., Riley, E.P., **Mattson**, S.N., and the CIFASD (2021). Correlation Between Results of an Automated Decision Tree Tool for Detecting FASD and Neuropsychological Outcomes. Presented at the Research Society on Alcoholism meeting, June 2021 (Virtual).

2. Courchesne, N.S., Hyland, M.T., Bernes, G.A., Krueger, A.M., Rockhold, M.N., Wozniak, Jones, K.L., Del Campo, M., Boys, C.J., **Mattson**, S.N. The impact of co-occurring alcohol and other drug exposure on child neuropsychological functioning. Presented at the Research Society on Alcoholism meeting, June 2021 (Virtual).

3. Sobolewski, C.M., Courchesne, N.S., Hyland, M.T., Bernes, G.A., Krueger, A.M., Rockhold, M.N., Wozniak, J.R., Jones, K.L., Del Campo, M., Boys, C.J., **Mattson**, S.N., & the CIFASD. (2021). Adaptive, externalizing, and internalizing behavior of children with prenatal alcohol exposure: A comparison of three parent-report questionnaires. Presented at the Research Society of Alcoholism meeting, June 2021 (Virtual).

4. Sobolewski, C.M., Courchesne, N.S., Hyland, M.T., Bernes, G.A., & **Mattson**, S.N. (2021). Differences in behavior based on IQ and prenatal alcohol exposure. Presented at the 101st annual Western Psychological Association Conference, April 2021 (Virtual).

5. Sobolewski, C.M., Courchesne, N.S., Hyland, M.T., Bernes, G.A., Jahan, T., **Mattson**, S.N., & the CIFASD. (2021). Externalizing but not Internalizing Behavior Ratings of Youth with Prenatal Alcohol Exposure Vary Based on the Rater's Relationship to the Subject. Presented at the 48th annual Society for Advancement of Chicanos/Hispanics and Native Americans in Science National Diversity in STEM (NDiSTEM) Conference, October 2021 (Virtual).

6. Poth, L.D. and **Mattson**, S.N. (2020). Language and Communication Abilities in Adolescents with Fetal Alcohol Spectrum Disorders. Presented at the International Neuropsychological Society Annual Meeting, San Diego, February 2021. (Virtual).

7. **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. Submitted for presentation at the Research Society on Alcoholism meeting, New Orleans, June 2020. (Conference Cancelled).

8. 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. Submitted for presentation at the Research Society on Alcoholism meeting, New Orleans, June 2020. (Conference Cancelled).

9. 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. Submitted for presentation at the Research Society on Alcoholism meeting, New Orleans, June 2020. (Conference Cancelled).

10. 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.

11. Wetherill, L., Nudelman, K., Schwantes-An, T-H, Boes, J, Parnell, SE, Abreu, M., Coles, C., Jones, K., Kable, J., Kan, E., Lai, D., Sowell, E., Wozniak, J., Riley, E.P., **Mattson**, S.N., Foroud, T., and the CIFASD (2019). Rare variants in 3 genes are associated with brain volume and executive function measures in prenatally alcohol exposed individuals. Presented at the Research Society on Alcoholism meeting, Minneapolis, June 2019. doi: 10.1111/acer.14058, Published online 23 May 2019. https://onlinelibrary.wiley.com/doi/10.1111/acer.14058

12. Kable, J.A., **Mattson**, S.N., Coles, C.D., and the CIFASD (2019). Changes in oxygenation of the prefrontal cortex predict memory errors in children with FASD. Presented at the Research Society on Alcoholism meeting, Minneapolis, June 2019. doi: 10.1111/acer.14058, Published online 23 May 2019. https://onlinelibrary.wiley.com/doi/10.1111/acer.14058

13. Inkelis, S.M. and **Mattson**, S.N. (2019). Age-related differences in measures of attention and sluggish cognitive tempo in children with histories of prenatal alcohol exposure. To be presented at the International Neuropsychological Society Meeting, February 2019.

14. Doyle*, L.R., Chambers, C.D., Jones, S.N., **Mattson**, S.N., and the CIFASD. (2018). Validation of a decision tree for clinical identification of children affected by prenatal alcohol exposure in a low-risk sample. Presented at the Research Society on Alcoholism meeting, San Diego, June 2018. doi:

10.1111/acer.13747, Published online 31 May 2018. https://onlinelibrary.wiley.com/doi/full/10.1111/acer.13747

15. Inkelis, S.M., Moore, E.M., **Mattson**, S.N., Riley, E.P. (2018). Predicting prenatal alcohol exposure histories using measures of attention and activity. Presented at the Research Society on Alcoholism meeting, San Diego, June 2018. doi: 10.1111/acer.13747, Published online 31 May 2018. https://onlinelibrary.wiley.com/doi/full/10.1111/acer.13747

16. Carvalho, R., **Mattson**, S.N., Riley, E.P., Moore, E.M. (2018). Cognition measured with NIH Toolbox in children with histories of heavy prenatal alcohol exposure. Presented at the Research Society on Alcoholism meeting, San Diego, June 2018. doi: 10.1111/acer.13747, Published online 31 May 2018. https://onlinelibrary.wiley.com/doi/full/10.1111/acer.13747

17. Wetherill, L., **Mattson**, S.N., Foroud, T., Goodlett, C., and the CIFASD. (2018). Effect of prenatal alcohol exposure and parental alcohol dependence on risk of externalizing disorders in COGA and CIFASD samples. Presented at the Research Society on Alcoholism meeting, San Diego, June 2018. doi: 10.1111/acer.13747, Published online 31 May 2018. https://onlinelibrary.wiley.com/doi/full/10.1111/acer.13747

18. Cheney*, D.C., Doyle*, L.R., Coles, C.D., Kable, J.A., May, P.A., Sowell, E.R., Jones, K.L., Riley, E.P., **Mattson**, S.N. and the CIFASD. (2017). Development and validation of a risk score that correlates with neurobehavioral effects of prenatal alcohol exposure. Presented at the Research Society on Alcoholism meeting, Denver, June 2017. doi: 10.1111/acer.13391, Published online 31 May 2017. http://onlinelibrary.wiley.com/doi/10.1111/acer.13391/full

19. Doyle*, L.R., Coles, C.D., Kable, J.A., May, P.A., Sowell, E.R., Jones, K.L., Riley, E.P., **Mattson**, S.N. and the CIFASD. (2017). Relation between adaptive function and iq among youth with histories of heavy prenatal alcohol exposure. Presented at the Research Society on Alcoholism meeting, Denver, June 2017. doi: 10.1111/acer.13391, Published online 31 May 2017. http://onlinelibrary.wiley.com/doi/10.1111/acer.13391/full

20. Infante, M.A., Moore, E.M., Bischoff-Grethe, A., Tapert, S.F., **Mattson**, S.N., and Riley, E.P. (2017). Spatial working memory in children with heavy prenatal alcohol exposure: a multimodal imaging approach. Presented at the Research Society on Alcoholism meeting, Denver, June 2017. doi: 10.1111/acer.13391, Published online 31 May 2017. http://onlinelibrary.wiley.com/doi/10.1111/acer.13391/full

21. Inkelis, S.M., Thomas, J.D., Coles, C.D., Kable, J.A., Wozniak, J.R., Riley, E.P., **Mattson**, S.N., and the CIFASD (2017). The relationship between sleep problems and behavioral functioning in 5-year-olds with prenatal alcohol exposure. Presented at the Research Society on Alcoholism meeting, Denver, June 2017. doi: 10.1111/acer.13391, Published online 31 May 2017. http://onlinelibrary.wiley.com/doi/10.1111/acer.13391/full

22. Moore, E.M., Haven, S., Gonzalez*, G., Murphy, C., Jones, K.L., **Mattson**, S.N., Giedd, J., and Riley, E.P. (2017). Impaired olfactory threshold detection in children with FASD. Presented at the Research Society on Alcoholism meeting, Denver, June 2017. doi: 10.1111/acer.13391, Published online 31 May 2017. http://onlinelibrary.wiley.com/doi/10.1111/acer.13391/full

Principal Investigators: Scott Parnell and Johann Eberhart
Institutions: University of North Carolina and University of Texas
CIFASD4 Project Title: Exploring the Genetics of FASD in Complementary Mouse and Fish Models
Grant Number: U01 AA021651

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. During this period we have examined sex-specific effects in our most often used strain of mice, the C57BL/6J stain. At the same time, but have begun a transcriptomic comparison between males and females of this strain. We have probed the role of *Htt*, *Efcab7*, *Kif3a*, and *Irf3* in potential gene/alcohol interactions, and have initiated a transcriptomic analysis of the Htt mice. We have been probing some of the Collaborative Cross (CC) founder strains (the C57BL/6J is one of the eight founder strains) for potential differences in susceptibility to prenatal alcohol exposure. Based on some of these data described below, we are in the process of genotyping all of our transgenic mice to determine their various background strains. We have performed phenotypic analyses of ethanol-treated zebrafish *nnt* mutants. We have performed RNA-seq on ethanol-treated and untreated *nnt* mutant and wild-type embryos for comparison to the mouse data that we have collected. We have examined the influence of the mTOR pathway on ethanol teratogenesis in zebrafish.

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.

3) Significant Results. We have demonstrated a significant gene/alcohol interaction of *Htt*, *Efcab7*, *Kif3a*, but not *Irf3*. We were expecting the Irf3 KO mice to be protected against the effects of alcohol relative to the Irf3 wild-type littermates, but interestingly, we saw very few effects in any of these mice suggesting a protective background strain in these transgenics. We are not aware of their exact background strain, but we are in the process of genotyping them to determine this and we suspect they are some variation of the C57/129S strains. At the same time we were doing these experiments, we were starting on the CC founder mice, specifically the 129S1/SvlmJ strain and discovered that these mice are completely resistant to the effects of a gastrulation-stage

alcohol exposure. This is especially exciting given our previous data in p53 KO mice demonstrating that the background strain was exceptionally vulnerable to alcohol (twice that of the C57 mice). Subsequent findings demonstrated the background strain of these transgenic mice is a mixed C57/129S2 background. Subsequent transcriptomic and genomic analyses should reveal key genes responsible for these small genetic changes, but huge differences in susceptibility.

We have found that apoptosis is elevated in ethanol-treated *nnt* mutants and are currently furthering these characterization by examining proliferation and patterning. We are also performing transgenic analyses to determine the effect of ethanol on neural crest cells and the endoderm in *nnt* mutants. We have just obtained our RNA-seq reads and will begin differential gene expression analyses. We are functionally characterizing the influence of ethanol on the mTOR pathway by translational profiling and analysis of autophagy.

4) Key Outcomes and Other Achievements. The data generated in the studies described here will not only provide numerous candidate genes that can be tested further for potential gene/alcohol interactions, but also will lay the groundwork for future NIAAA grant proposals concerning the Collaborative Cross mice.

Additional Questions

Final Year. What do you plan to do between now and May 2022* to accomplish your CIFASD4 goals and objectives? *For those with UH2s funded in 2021, what are your goals for year two of your support?

Finish up the experiments started above and publish them.

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

Discussed candidate genes with the Human Genetics component.

Administrative Supplements. For this reporting period (June 2021 to present), please list any Administrative Supplement associated with this award and a sentence or two about what it entails and its current status. n/a

Publications/Abstracts

Publications [Accepted & In Press]

Fish EW, Tucker SK, Peterson, RL, Eberhart JK, Parnell SE. 2021. Loss of Tumor Protein 53 protects against alcohol-mediated facial malformations in mice and zebrafish. Alcohol Clin Exp Res.

Boschen KE, Ptacek TS, Berginski ME, Simon JM, Parnell SE. 2021. Transcriptomic analyses of gastrulation-stage C57BL/6J and C57BL/6NHsd mouse embryos with differential susceptibility to alcohol. Dis Model Mech. 14(6):dmm049012.

Sidik A, Groves D, Buckley DM, Kirby HG, Sun S, Eberhart JK. 2021. Exposure to ethanol leads to midfacial hypoplasis in a zebrafish model of FASD via indirect interactions with the Shh pathway. BMC Biology 19(134)

Everson JL, Batchu R, Eberhart JK. 2020. Multifactorial genetic and environmental hedgehog pathway disruption sensitizes embryos to alcohol-induced craniofacial defects. Alcohol Clin Exp Res. 44(10).

Swartz ME, Lovely CB, McCarthy N, Kuka T, Eberhart JK. 2019. Novel ethanol-sensitive mutants identified in an F3 forward genetic screen. Alcohol Clin Exp Res. 44(1).

Buckley DM, Sidik A, Kar RD, Eberhart JK. 2019. Differentially sensitive neuronal subpopulations in the central nervous system and the formation of hindbrain heterotopias in ethanol-exposed zebrafish. Birth Defects Res. 111(12).

Fernandes Y, Rampersad M, Jones EM, Eberhart JK. 2019. Social deficits following embryonic ethanol exposure arise in post-larval zebrafish. Addict Biol. 24(5)

Fernandes Y, Rampersad M, Eberhart JK. 2019. Social behavioral phenotyping of the zebrafish casper mutant following embryonic alcohol exposure. Behav Brain Res. 356:46-50.

Tang X, Eberhart JK, Cleves MA, Li J, Li M, MacLeod S, Nembhard WN, Hobbs CA. Sci Rep. 8(1).

Publications [In Preparation & Submitted]

None

Poster Abstracts and Presentations

Parnell SE, Boschen KE, Fish EW. 2021. Discovering Candidate Genes in Mice That Contribute To FASD: Involvement of Growth Factor and Primary Cilia Pathways. Alcohol Clin Exp Res. 45(S1):29a. https://doi.org/10.1111/acer.14617.

Boschen KE, Fish EW, Mendoza-Romero HN, Eberhart JK, Parnell SE. 2021. Genetic contribution of oxidative stress and apoptosis pathways to prenatal alcohol sensitivity. Alcohol Clin Exp Res. 45(S1):64a. https://doi.org/10.1111/acer.14617.

Steensen MC, Boschen KE, Hunter CE, Parnell SE. 2021. Prenatal Alcohol Affects Incidence of Eye Defects in Htt Mutant Mice in a Sex and Dose-Dependent Manner. Alcohol Clin Exp Res 45(S1):223a. https://doi.org/10.1111/acer.14628.

Cannizzo MD, Fish EW, Parnell SE. 2021. Kif3a: A primary cilia gene associated with susceptibilities to prenatal alcohol exposure. Alcohol Clin Exp Res 45(S1):161a. https://doi.org/10.1111/acer.14628.

Principal Investigators: Christie Petrenko and Cristiano Tapparello
Institution: University of Rochester
CIFASD4 Title: Development and Evaluation of an Evidence-Based Mobile Health Caregiver Intervention for FASD
Grant Number: U01 AA026104

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 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) comparing intervention and waitlist groups.

Accomplishments

1) Major Activities.

Submission of CIFASD5 Renewal: Our project entitled "Leveraging Technology to Increase Quality of Life for FASD Across the Lifespan" was selected for the CIFASD5 package and was submitted in August 2021.

Best-test Manuscript (Aim 1): Our manuscript submitted in February 2021 was reviewed and offered a revise and resubmit in August 2021. Revisions were submitted in September and the manuscript was accepted for publication in October 2021.

Feasibility Trial (Aim 2): Baseline data collection for Android users was initiated 1/26/21 and follow-up assessments and interviews were completed 7/30/2021. Out of a possible 39 users enrolled, 33 completed baseline assessments and 22 completed follow-up surveys. Qualitative and quantitative analyses for both iOS and Android users are underway. Please see preliminary findings in section 3.

Doctoral student Carson Kautz-Turnbull recently presented at the 2021 ESBRA meeting (October 2021) on baseline data from the feasibility trial investigating adverse childhood experiences (ACEs) in 87 children in our sample. Ms. Kautz-Turnbull received a young investigator award for this work. See findings in section 3.

Preparation for RCT (Aim 3): We completed the following activities: 1) *measure development*: based on feasibility trial results, revised a measure of FASD knowledge and developed a new measure of adaptive behavior; 2) *recruitment development*: identified clinics across US to engage newly diagnosed families, worked with our CTSI to create engaging recruitment materials, started a newsletter (Thrive Lifelong; see www.fmfconnect.com), prepared REDCap database infrastructure; 3) *regulatory*: obtained IRB approval, registered study on ClinicalTrials.gov, updated human subjects and good clinical practice certifications; and 4) *developed coaching arm*: defined coaching role, hired and trained coaches, and prepared sample responses.

Dr. Tapparello completed extensive programming updates to the iOS app. The iOS app has been updated to support devices running iOS 15 (released by Apple in September 2021), and to improve the interaction with the Cloud functionalities hosted on Amazon Web Services (AWS). These updates allow for quick transitions between the development and the production environment as well as to more easily enroll and distribute the app to new users during the upcoming RCT. In addition, we have: 1) transitioned the app video library to a new AWS streaming service that dynamically adjusts the video quality to the user Internet service; 2) implemented a new system to track the user interactions with the app; 3) connected the tracking system to a companion website that allows the research team to track users' progress and utilization in real time; 3) implemented a push notification system to notify users of new posts and comments on the Family Forum; and 4) implemented functionalities (like, e.g., direct messaging and notifications) to support the RCT coaching arm. During the RCT, the tracking system and the companion website will also be used by the coaches to monitor their assigned participants.

Presentations: Our team has been active in disseminating research findings, with 4 scientific presentations in the last 6 months. Dr. Petrenko has also been featured on the FASD Success podcast with Jeff Noble and she

and Dr. Carmichael Olson have given other presentations to trainees and lay audiences on CIFASD work.

FMF Connect Teacher Companion Website: Ms. Kautz-Turnbull resubmitted her Diversity F31 NRSA in August 2021 to develop and pilot a Teacher Companion Website for FMF Connect and received a score of 18 (7%ile). If funded, the fellowship will begin April 2022. She is currently analyzing 23 teacher interviews completed in April – June 2021.

2) Specific Objectives.

Aim 1: Using mixed methods analysis across two rounds of beta-test data, evaluate: 1) how well the app works from a technological standpoint, and 2) the user experience to identify any needed refinements. Completed: R&R August 2021; manuscript was accepted for publication October 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: Android data collection complete July 2021. Preliminary data presented below.

Aim 3: Conduct an RCT with three groups (FMF Connect + Coaching, FMF Connect, Waitlist Control) to evaluate relative efficacy of the FMF Connect app with or without coaching. Progress: Prepared to begin recruitment at the beginning of December 2021.

3) Significant Results.

ACEs in young children with FASD and effect on behavior problems (ESBRA, 2021): Participants included 87 caregivers in the feasibility trial who reported on ACEs and child behavior problems (Eyberg Child Behavior Inventory; ECBI) at baseline. Data were analyzed using Pearson correlational analyses and linear regression. On average, caregivers endorsed 3.10 (SD=2.99) ACEs, which is almost twice that in the general population (1.56; Giano, Wheeler, & Hubach, 2020). Correlational analyses revealed that current foster care placement was significantly associated with a higher total ACEs score (r=.25, p<.05). Total ACEs score was not significantly associated with child age, household income, household size, or caregiver type other than foster parent. Mean ECBI Intensity T-score was 69.94 (SD=9.17), which is in the clinical range. The number of ACEs a child had experienced significantly predicted child behavior problems (b=.33, p<.01), controlling for child age, household size, caregiver type, and household income. This underscores the importance of screening for ACEs and providing appropriate support for children with FASD or PAE who have experienced adverse childhood events, especially those in foster care. A manuscript detailing these findings is in preparation.

Feasibility trial preliminary findings (Aim 2): Several aspects of feasibility were assessed. First, we continued to assess intervention feasibility, including technological functioning of the app and acceptability to users. The iOS app continues to be very stable across beta-test and feasibility trials; the Android app has had more technological difficulties, likely related to less standardization across Android devices and versions of operating system. Across both device types, users continue to express high satisfaction with the app (see preliminary quantitative and qualitative findings below for additional details). Next, we considered trial feasibility. We had 183 participants initiate the consent process, showing high interest in the study and feasibility of recruitment. We had broad reach across the US; areas for additional targeted outreach were identified for the future RCT. 105 participants were deemed eligible for the trial, 26 ineligible, and 52 had incomplete files. We identified several inefficiencies to improve participant completion for the RCT. We also examined attrition data: 82% of eligible participants completed baseline assessments. Of these, 80% installed the app. 52 participants completed follow-up assessments. Various strategies were attempted to engage participants to encourage measure completion. Time was a major barrier for families. We have subsequently worked to try to streamline measures for the RCT. Quantitative data below document that measures are sensitive to change. Finally, we examined caregiver app usage data. The usage tiers identified in beta-testing remained a good fit for characterizing iOS feasibility usage; Android usage data is in progress. 20% of iOS users completed all 12 Learning Modules; 50% completed level 1 modules (1-4). Based on usage patterns we designed the RCT to have 3 timepoints: baseline (T1), 6 weeks (T2), and 12 weeks (T3).

Quantitative Findings: A total of 52 caregivers across iOS and Android trials completed both pre- and post-intervention assessments. Mean overall app quality ratings on the User Version of the Mobile Application Rating Scale (uMARS) were 4.07 (SD=0.47) out of 5. Consistent with the primary purpose of the app, Information scores received the highest ratings (Engagement=3.71; Functionality=4.07; Aesthetics=4.10; Information=4.44). iOS users rated the FMF Connect app significantly higher than Android users (iOS overall=4.24, Android overall=3.86; p=.004). Repeated measures ANOVAs, controlling for type of device, identified statistically significant reductions in child behavior problems (ECBI intensity T1=69.20, T2=65.62, p<.001), increased FASD knowledge (K&A T1=34.44, T2=35.38, p=.013), and improvements in caregiver parenting self-efficacy (PSOC

T1=22.04, T2=20.56; *p*=.005) and satisfaction (PSOC T1=32.90, T2=34.02, *p*=.049). Effect sizes were in the small to medium range (d= 0.17 to 0.43); device type did not moderate any effects. Although this feasibility trial did not include a control group, the similarity of findings across iOS (Spring 2020) and Android (Spring 2021) trials suggests a possible intervention signal. The RCT is designed to robustly test efficacy.

Qualitative Findings: Systematic thematic analysis is underway. Emerging preliminary themes on the user experience are similar to those identified during beta-testing. Participants continue to be very positive about the app and view it as a beneficial tool for caregivers raising children with FASD. They emphasize that FASD is a complex/confusing diagnosis and that there is a lack of information out there, resulting in caregivers feeling isolated. Information in the app and opportunity to connect with other parents in the Family Forum are motivators for app use. Participants provided positive evaluations of a number of app features, and especially like the Tip of the Day. There were mixed views regarding the daily self-care ratings. Barriers to use included technology problems (more common for Android) and the COVID-19 pandemic. Additional recommendations were offered for app refinement, including content (e.g., navigating pandemic with kids, teacher resources) and app functions (e.g., notifications, joint account with partner, use for multiple children). Participants commonly used the app whenever they had downtime, and after their kids went to bed. Many expressed that they would like to continue using the app in the future.

4) Key Outcomes and Other Achievements.

Results across beta-test and feasibility trials indicate FMF Connect app is feasible and acceptable to caregivers. Theory- and data-driven refinements have been implemented for the RCT launching at the start of December. Our team has productive over the last 6 months, including 1 new accepted manuscript and 4 presentations.

Additional Questions

Final Year. What do you plan to do between now and May 2022* to accomplish your CIFASD4 goals and objectives? *For those with UH2s funded in 2021, what are your goals for year two of your support?

We plan to complete qualitative and quantitative analyses for the feasibility trial and prepare a manuscript for publication in the first quarter of 2022. We will be initiating the RCT at the start of December and expect to complete the trial in Spring 2022. We will then analyze data and prepare a manuscript for publication.

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 2021, our project has referred XX participants from our FMF Connect trial to the DiG study. In collaboration with IU, we were able to modify the DiG protocol for our site to allow us to re-contact participants who completed Dr. Petrenko's Tuning in to Kids (TIK) clinical trial to allow in person data collection of saliva and 2D and 3D photos. We originally had approval to approach families to collect this data in coordination with TIK research visits, but this was interrupted due to the COVID-19 pandemic. TIK research staff are in the process of re-contacting families to determine if they are willing to schedule visits. Providers in our FASD Diagnostic Clinic have also been routinely using the Morpheus Q app as part of all in-person diagnostic evaluations to gather clinical data on the utility and concordance of the app to manual measurements. We will be reaching out to other CIFASD PIs shortly to aid in recruitment for the FMF Connect RCT.

Administrative Supplements. For this reporting period (June 2021 to present), please list any Administrative Supplement associated with this award and a sentence or two about what it entails and its current status. N/A

Publications/Abstracts

Publications [Accepted & In Press]

Petrenko, C. L. M., Kautz-Turnbull, C., Roth, A., Parr, J, Tapparello, C., Demir, U., Olson, H. C. (in press). The Families Moving Forward Connect mobile health intervention for caregivers of children with fetal alcohol spectrum disorders: Findings from beta-testing within a systematic user-centered design approach. *JMIR Formative Research.*

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. (2021). Partner influence as a factor in maternal alcohol consumption and depressive symptoms, with subsequent effects on infant neurodevelopmental outcomes. *Alcoholism: Clinical & Experimental Research, 45,* 1265-1275. PMCID: PMC8254755

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 (2020): e14721. PMCID: PMC7171567

Publications [In Preparation & Submitted]

Kautz-Turnbull, C., Rockhold, M., Olson, H.C., & Petrenko, C.L.M. (in preparation). Adverse childhood experiences (ACEs) in young children with fetal alcohol spectrum disorder (FASD) and effect on behavior problems.

Kautz-Turnbull, C., Rogge, R., & Petrenko, C.L.M. (in preparation). Reasons for Children's Behavior: Development and Validation of a New Measure of Parental Attributions.

Poster Abstracts and Presentations

Petrenko, C.L.M., Cole, L., & Kautz-Turnbull, C. (2021). Leveraging technology to increase access to diagnosis and fetal alcohol spectrum disorder (FASD)-informed care across the lifespan. Australasian Professional Society on Alcohol and Other Drugs FASD Special Interest Group meeting. November 7, 2021.

Petrenko, C. L. M. (2021). Development and evaluation of digital and mobile health interventions (eHealth) for FASD. Virtual Presentation in symposia "Diagnosis and Interventions in FASD: From Genes to eHealth." European Society for Biomedical Research on Alcohol, Timisoara, Romania, October 2021.

Kautz-Turnbull, C., Olson, H.C., & Petrenko, C.L.M. (2021, October). Adverse childhood experiences (ACEs) in young children with fetal alcohol spectrum disorder (FASD) and effect on behavior problems. Virtual Presentation at Young Investigator Symposium, European Society for Biomedical Research on Alcohol, Timisoara, Romania.

Roth, A., Kautz-Turnbull, Petrenko, C.L.M., Parr, J., Tapparello, C., Olson, H.C. (2021). Preliminary findings from the feasibility trial of Families Moving Forward Connect mobile health intervention for caregivers of children with FASD. *Alcoholism: Clinical and Experimental Research*, *45,* 389. Poster presentation at the 43rd Research Society on Alcoholism, June 2021.

Petrenko, C. L. M., & Kautz-Turnbull (2021). From surviving to thriving: Advancing interventions and supports for FASD. Invited virtual presentation to the National Organization on Fetal Alcohol Syndrome Affiliates, May 20, 2021.

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. 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 Investigators: Alison Noble and Michael Suttie
Institution: University of Oxford
CIFASD4 Project Title: Image Analysis of Neurofacial Effects of Prenatal Alcohol Exposure
Grant Number: U01 AA014809

Specific Aims

1. Automated screening of facial images for effects of prenatal alcohol exposure with potential for online and mobile device use and integration of genetic, behavioural and cognitive data.
2. Improved analysis of face-neurocognitive-alcohol interactions.
3. Fetal ultrasound analysis to detect facial, cranial and neural effects of prenatal alcohol exposure with neonatal follow-up.

Accomplishments

Major Activities.

Facial Anatomical Landmark Detection

A primary goal for CIFASD4 was the automation of facial analysis. The assessment of facial form using statistical shape analysis and assessment using clinically relevant measurements such as PFL both require anatomically accurate landmark placement. With access to large amounts of data from the CIFASD consortium and PASS network, we developed and applied sophisticated machine learning techniques to 3D images to accurately place landmark points. Over the last few years, we have disseminated these results in imaging-based conferences and more recently developed FASD specific models recently published in an IEEE journal (Fu et al., 2021).

Clinical Translation: FaceScreen Tool

Technical development of our screening software 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 the University of Oxford), which can be accessed through API calls. In addition, we have built a user-friendly web-based front end for users to test. Currently, 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. A spare set of facial landmarks is required for the Canfield systems, but we have put in significant work to translate and implement reliable automated methods from the technical developments recently published. Once an image is uploaded and passes through the automated landmark pipeline, selected facial analysis can be generated to show facial dysmorphism including, PFL, control-FAS classification testing, lip-philtrum smoothness estimation, mid-facial flatness, and an interactive 3D heatmap showing normalized differences compared to age-sex matched controls. The past few months have seen significant progress with this task, and the focus has been on integrating automated landmarking and robust pipelines for automated analysis. In addition, we have been working to facilitate data storage and access and refining the front end interface.

Study: Objective, Accurate Identification of FASD Associated Facial Dysmorphology using 3D Imaging

Throughout CIFASD4, we have been working in close collaboration with Dr Raja Mukherjee, who operates one of only two FASD clinics in England. In this time, he and his team have managed to collect 3D data for nearly 130 patients in combination with 2D assessments using the 2D FAS Photographic Tool and the 4-digit code. This data, in combination with CIFASD data, has provided us with an opportunity to validate and assess some of the automated techniques developed this year for 3D facial analysis and clinical evaluation. It also allows for a comparison with the 2D FAS assessment tool, which is currently the only clinically available software to facilitate the evaluation of FASD associated facial dysmorphism. Machine learning methods developed and published this year for automated landmarking are being utilized in this analysis, and we are focused on validating them and comparing them with 2D manual measurements. In addition, we are utilizing existing published CIFASD FASD models for discrimination testing to determine the best methodology for identification across the FASD spectrum. In this study, we investigate the concordance between 2D and 3D measurement methods, identification and prevalence of minor facial anomalies using 3D analysis and compare the clinical diagnostic outcome with the 4-digit code. In doing so, we have also further refined automated landmark detection models to identify precise eye points.

Collaboration with University of Leuven: Infant Craniofacial Replication Study

We have recently established a new collaboration with Dr Harry Matthews from Prof Peter Claes' group at the University of Leuven. Dr Harry Mathews and Dr Suttie have been in close contact recently to undergo a replication study of the 2017 paper by Muggli et al., which focused on investigating the association between

prenatal alcohol exposure and craniofacial shape of children at 12 months of age. In this study, the investigators examined the association between dose, frequency, and timing of prenatal alcohol exposure and craniofacial differences from 3D image analysis. This investigation focuses on exposure levels classified as low, moderate to high, or binge-level in the first trimester or throughout pregnancy and concluded that differences were seen at all levels of exposures regardless of 1st trimester occurrence. After some discussion with Dr Mathews, we concluded that data obtained for CIFASD4 from the PASS cohort had coincident data collection methodology, exposure information, and imaging timepoints used in the Muggli et al. 2017 paper. Replication is essential, and we see this as an opportunity to both utilize data available and already processed and validate these findings on a different cohort.

Personelle Changes

Recently, we have had a new starter Dr Mingze Yuan, who will be focusing on enhancing some of the technical aspects of the project. This post was due to be filled almost 12 months ago, but due to a candidate dropping out at the last minute, followed by a failed recuiting round, we were only able to fill it in October 2021 on the third round of recruitment. However, Dr Yuan has integrated well into the team and has already produced some promising developments for our translational work.

Additionally, we have recently been fortunate enough to re-hire Dr Ralf Huesler, who previously worked as a lead developer for the FaceScreen software. Rejoining the project has been hugely beneficial as we complete some of the online and mobile device integration of our clinical software and test on UK FASD Clinic and CIFASD data.

1) Specific Objectives.

Clinical Translation

- Test FaceScreen analysis tools which utilize dense surface modelling onto an accessible and secure server
- Develop an enhanced web-based front end and test database-driven access for image analysis
- Integrate and test automated landmarking pipelines on our cloud server for analysis and measurement feedback

Infant Craniofacial Replication Study

- Set up collaboration with Dr Harry Mathews (University of Leuven)
- Image analysis of PASS infant data to recapitulate Muggli et al. 2017

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.

2) Significant Results.

Automated Landmarking for Cardinal Feature Detection: PFL

Automated landmarking techniques designed using regularized transfer learning were published in Fu et al. 2021. In this paper, we showed the accuracy of our methodology compared to similar techniques to perform significantly better for full-face landmarking. These models were trained for the purpose of accurate identification of anatomically accurate landmark points, and our approach is strengthened by working with FASD specific training data. After this publication, we have taken these methods further and developed eye-specific models for a refined automated measurement of PFL. Using over 2800 manually annotated images from the CIFASD and PASS cohorts, we were able to train models for the purpose of PFL measurement, and on a test set of nearly 300 images, were able to gain accurate measurements with no significant difference compared to manual placement. In this test, we achieved a mean error of just 0.235mm (+/- 0.69 std) with minimum and maximum errors of -3.17mm and 2.56mm respectively. Some examples of the degree of accuracy achieved can be seen in Figure 1.

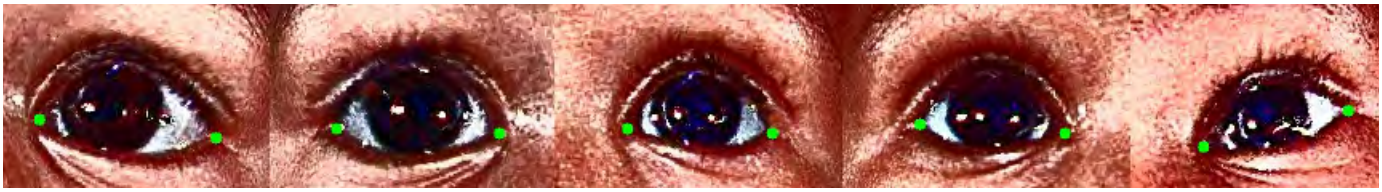


Figure 1 Fully automated placement of inner and outer canthi points to calculate PFL from 3D images.

Lip-Philtrum

We have been testing and refining lip-philtrum models to achieve greater cohesion with clinical assessment. In our 3D (DSM) model of 470 Caucasian subjects recruited by CIFASD (alcohol exposed = 2020), we observed those with a smooth philtrum to primarily be scored in clinical assessment as a 4, with only 5% being a 5, and similarly a control-like philtrum was primarily scored a 3 with only 18% scoring a 2, and only a single individual with a score of 1. Using DSMs of 3D shape, we are able to perform multi-class discrimination testing to determine which of the five philtrum classifications an individual is closest to; however, given the low numbers, this is not an option. Instead, we group 1-3 as not smooth and 4-5 as smooth and apply binary classification methods. Results using closest mean classification were impressive, achieving an accuracy of 0.9 (AUC), which slightly improved with more sophisticated techniques (support vector machines 0.92; linear discriminate analysis 0.91). Further work to improve and integrate these models into our FaceScreen software is currently underway.

Objective, Accurate Identification of FASD Associated Facial Dysmorphology using 3D Imaging

Results from our study utilizing automated methods for FASD detection have shown:

i. PFL measurements derived from this fully automated pipeline correlate with; manually placed 3D landmark placement, clinical measurements using a ruler, and 2D measurements. We observe greater cohesion between automated and manual 3D placement and show a significant difference to those taken using a ruler.
ii. Automated classification of FAS individuals was not in agreement with the clinical diagnosis using the 4-digit code.
iii. Subtle FASD facial dysmorphism, namely micrognathia and midfacial hypoplasia, were identified in a significantly larger number of alcohol-exposed individuals compared to controls
iv. Philtrial smoothness and a thin upper-lip vermillion were in agreement with face-to-face dysmorphology examinations. With discrimination testing between smooth and grooved philtrums achieved 0.9 classification accuracy (AUC).

2D Facial Analysis

We have built shape models of facial differences from 2D images collected by CIFASD members. Our focus was to determine correlations between facial form and WES data collected by the DiGFASD study. Unfortunately, no hits were found, and 2D models were ineffective in this case. We will continue to process the 2D data collected to determine if alternative methods could yield more significant findings.

4) Key Outcomes and Other Achievements.

Additional Questions

Final Year. What do you plan to do between now and May 2022* to accomplish your CIFASD4 goals and objectives? *For those with UH2s funded in 2021, what are your goals for year two of your support?

Primarily we will focus on deliverables before the end of the phase. We are aiming to:

- Publish on young populations from both the PASS dataset and UK neonatal COFASD cohort collected in CIFASD4
- Submit automated clinical validation study (targeting ACER)
- Complete replication study in collaboration with the University of Leurven
- Continue developments of the FaceScreen Server to distribute to CIFASD members for use

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.

Administrative Supplements. For this reporting period (June 2021 to present), please list any Administrative Supplement associated with this award and a sentence or two about what it entails and its current status.

None

Publications/Abstracts

Publications [Accepted & In Press]

Fu, Z., Jiao, J., Suttie, M., & Noble, J. A. (2021). Facial Anatomical Landmark Detection using Regularized Transfer Learning with Application to Fetal Alcohol Syndrome Recognition. 2194(c), 1–11. https://doi.org/10.1109/JBHI.2021.3110680

Schölin, L., Mukherjee, R. A. S., Aiton, N., Blackburn, C., Brown, S., Flemming, K. M., Gard, P. R., Howlett, H., Plant, M., Price, A. D., Shields, J., Smith, L. A., Suttie, M., Zammitt, D. C., & Cook, P. A. (2021). Fetal alcohol spectrum disorders: An overview of current evidence and activities in the UK. Archives of Disease in Childhood, 106(7), 636–640. https://doi.org/10.1136/archdischild-2020-320435

Fu, Z., Jiao, J., Suttie, M., & Noble, J. A. (2020). Cross-Task Representation Learning for Anatomical Landmark Detection. arXiv:2009.13635v1 [cs.CV] 28 Sep 2020

Ruobing Huang, Michael Suttie, J. Alison Noble. An Automated CNN-based 3D Anatomical Landmark Detection Method to Facilitate Surface-Based 3D Facial Shape Analysis. UNSURE/CLIP@MICCAI 2019: 163-171

Aiton N, Huang R, Fernandez R, Mills M, Suttie M (2019) Novel techniques for the analysis of face-brain morphology in babies and adolescents with prenatal alcohol exposure (PNAE). Archives of Disease in Childhood May 2019, 104 (Suppl 2) A79; DOI: 10.1136/archdischild-2019-rcpch.190

Aiton N, Suttie M, Ferguson A (2019) Identifying facial features associated with prenatal alcohol exposure in newborn infants using 2D and 3D imaging. Archives of Disease in Childhood May 2019, 104 (Suppl 2) A87-A88; DOI: 10.1136/archdischild-2019-rcpch.210

Basart, H., Suttie, M., Ibrahim, A., Ferretti, P., van der Horst, C. M. A. M., Hennekam, R. C., & Hammond, P. (2018). Objectifying micrognathia using three-dimensional photogrammetric analysis. Journal of Craniofacial Surgery, 29(8), 2106–2109. https://doi.org/10.1097/SCS.0000000000005056

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., Foroud, T., & Hammond, P. (2018). Combined Face–Brain Morphology and Associated Neurocognitive Correlates in Fetal Alcohol Spectrum Disorders. Alcoholism: Clinical and Experimental Research, 42(9), 1769–1782. https://doi.org/10.1111/acer.13820

Dou, X., Menkari, C., Mitsuyama, R., Foroud, T., Wetherill, L., Hammond, P., Suttie, M., Chen, X., Chen, S. Y., & Charness, M. E. (2018). L1 coupling to ankyrin and the spectrin-actin cytoskeleton modulates ethanol inhibition of L1 adhesion and ethanol teratogenesis. FASEB Journal, 32(3), 1364–1374. https://doi.org/10.1096/fj.201700970

Ruobing Huang, Weidi Xie, J. Alison Noble. VP-Nets : Efficient automatic localization of key brain structures in 3D fetal neurosonography. Medical Image Anal. 47: 127-139 (2018)

Ruobing Huang, J. Alison Noble, Ana I. L. Namburete. Omni-Supervised Learning: Scaling Up to Large Unlabelled Medical Datasets. MICCAI (1) 2018: 572-580

Fish, E. W., Wieczorek, L. A., Rumple, A., Suttie, M., Moy, S. S., Hammond, P., & Parnell, S. E. (2018). The enduring impact of neurulation stage alcohol exposure: A combined behavioral and structural neuroimaging study in adult male and female C57BL/6J mice. Behavioural Brain Research, 338(919), 173–184. https://doi.org/10.1016/j.bbr.2017.10.020

Suttie, M., Wetherill, L., Jacobson, S. W., Jacobson, J. L., Hoyme, H. E., Sowell, E. R., Coles, C., Wozniak, J. R., Riley, E. P., Jones, K. L., Foroud, T., & Hammond, P. (2017). Facial Curvature Detects and Explicates Ethnic Differences in Effects of Prenatal Alcohol Exposure. Alcoholism: Clinical and Experimental Research, 1–13. https://doi.org/10.1111/acer.13429

Publications [In Preparation & Submitted]

H Matthews, H Odendaal, Elliott AJ, M Suttie and the CIFASD. Association Between Prenatal Alcohol Exposure and Facial Dysmorphism in Infants. ***In preparation***

Z Fu, R Huesler, R Mukherjee, Jeff Wozniak, L Wetherill, T Foroud, S Mattson, P Hammond, M Suttie and the CIFASD. Objective, Accurate Identification of FASD Associated Facial Dysmorphology using 3D Imaging. ***In preparation***

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. ***In preparation***

Poster Abstracts and Presentations

Presentations

M Suttie, Z Fu (2021) Utilizing 3D Facial Analysis To Estimate The Prevalence Of Minor Facial Anomalies In FASD. European Society for Biomedical Research on Alcoholism

M Suttie. (2021) 3D Facial Imaging for the Identifcation of FASD. UK FASD Conference 2021.

M Suttie, P Hammond, N Aiton and the CIFASD (2019) Utilizing 3D Facial Analysis for the Early Identification of FASD Associated Facial Dysmorphism at Neonatal and Infant Stages. European Society for Biomedical Research on Alcoholism

M Suttie, P Hammond, R Huang and the CIFASD (2019). Identifying FASD Associated Facial Dysmorphology Using Automated Analysis of 2D and 3D Facial Imaging. Research Society on Alcoholism

M Suttie.(2019) Utilizing 3D Facial Analysis for the Early Identification of FASD Associated Facial Dysmorphism at Neonatal and Infant Stages. European Society for Biomedical Research on Alcoholism

R Huang, M Suttie, A Noble (2019) An automated CNN-based 3D anatomical landmark detection method to facilitate surface-based 3D facial shape analysis. The Medical Image Computing and Computer Assisted Intervention Conference, Shenzhen, China

N Aiton, M Suttie (2019) Changes In The Corpus Callosum In Newborn Infants With Prenatal Alcohol Exposure. 8th International Conference on Fetal Alcohol Spectrum Disorder

M Suttie (2019) 3D Facial Analysis for the Objective Identification of FASD Associated Facial Dysmorphology. 8th International Conference on Fetal Alcohol Spectrum Disorder

M Suttie & P Hammond. (2018). Utilizing 3D Imaging To Identify Minor Facial Anomolies And Ethnic Differences In The Effects Of Prenatal Alcohol Exposure. Research Society on Alcoholism

M Suttie, R Mukherjee, P Hammond (2017). Introducing 3D analysis into the Clinical Workflow for the Recognition of FASD Associated Facial Dysmorphism. European FASD Alliance Conference

Principal Investigator: Joanne Weinberg
Institution: University of British Columbia
CIFASD4 Project Title: Immune Dysregulation in FASD: Programming of Health and Neurobehavioral Outcomes
Grant Number: U01 AA026101

Specific Aims

Risk for adult diseases or disorders is influenced by prenatal and early life environmental experiences. Building on seminal studies by Barker and colleagues, who reported associations between low birth weight and biological risk for adult disease, support for the "Developmental Origins of Health and Disease" (DOHaD) hypothesis has grown to include a broader range of early life adversities and to extend beyond metabolic disorder as outcomes. This "developmental model" suggests that factors influencing feto-placental or early life development change gene expression and reset or program functional capacity, metabolic competence and responses to later environmental challenges. Of particular relevance, 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) have shown more severe and prolonged inflammation (adjuvant-induced arthritis) than that in controls following immune challenge, which appears to have its basis in immune dysregulation (pro-inflammatory bias) present from birth. These findings 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 the increased vulnerability to adverse health outcomes. Data on other neurodevelopmental disorders support this suggestion; alterations in maternal immune responses appear to program offspring immune function in both autism and schizophrenia. Our CIFASD Developmental Project (DP) is the first to identify links among maternal alcohol consumption, inflammation, and child outcomes; unique immune signatures in pregnant women were identified in association with both alcohol consumption and neurodevelopmental outcomes of their children. The proposed UO1 builds on these findings to examine immune profiles in pregnant women and children from birth to adulthood. This brings a significant new dimension to CIFASD, a focus on DOHAD/health outcomes, a critically important but relatively understudied area in the FASD field. Moreover, as the immune system plays a key role in brain development, aberrant immune/inflammatory mechanisms may also underlie FASD-associated neurobehavioral deficits that are well documented in the domains of neurocognition, self-regulation, and adaptive function. Our *working hypothesis* is that alcohol-induced dysregulation of immune/inflammatory function will be associated with adverse health, functional and adaptive outcomes, providing unique insight into factors underlying risk and resilience.

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

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

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

Accomplishments

1) Specific Objectives. During the past 6 months, our objectives were to move Aims 1 and 2 forward as much as possible. It has been challenging to have 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 data and blood samples are in the same situation and until recently have only been able to move forward in a limited manner. Nevertheless, we have made progress in a number of areas that move our project forward.

2) Major Activities and Significant Results.

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.

Collaboration with Dr. Tina Chambers' longitudinal study in Western Ukraine. We are working on a manuscript that examines cytokine profiles and outcome measures in *matched mother/child pairs* from the longitudinal study. Our data will not only assist us in determining the utility of immune parameters as possible biomarkers and predictors of alcohol-related neurobehavioral outcomes, but will also help to elucidate more directly the influences of the maternal immune milieu on child immune and neurobehavioral outcomes. [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*.] Data analysis is now almost complete and we expect to submit this manuscript in the next few months.

In parallel with Dr. Rajesh Miranda, we have just received an additional shipment of samples from children and mother/child pairs from Ukraine, thus significantly increasing the number of overlapping maternal and child samples between the Miranda lab and our lab, and providing the basis for a joint paper. Once the assays are completed, we will work with Miranda's group to assess whether the combined analysis of cytokines and miRNAs in maternal samples can serve as stronger/more accurate predictors of child neurodevelopmental trajectories, and in child samples can serve as stronger/more accurate biomarkers of PAE/FASD compared to either cytokines or miRNAs alone.

Child study in San Diego with Drs. Tina Chambers and Ken Jones. Children (ages 5-18) are being recruited from the San Diego FASD Research Subject Pool, Rady Children's Hospital. Data collected include diagnosis, demographic information and health outcomes, as well as assessment of plasma cytokine levels by our lab and miRNA levels by Dr. Rajesh Miranda. We have both just received a set of samples for analysis (n=11 ARND, 5 FAS, 10 PAE, 7 PFAS) and as above, we will work together to understand whether the combined analysis of two molecular markers can serve as stronger/more accurate biomarkers of PAE/FASD than either marker alone

For children in the San Diego FASD Research Subject Pool who were born in San Diego County, there is a Biorepository that contains blood spots taken from these children at birth as well as mid-gestation blood samples from their mothers. Dr. Chambers is investigating the possibility of obtaining blood spots and maternal blood samples to add to our analysis. This additional information would not only allow for a unique exploration of the immune trajectory of these children over time, but also provide validation cohorts for our previous maternal and child studies and for the investigation of how the maternal immune environment influences child outcomes.

Collaboration with Dr. Jeff Wozniak. Blood samples (67 samples from 34 unique subjects) from Dr. Wozniak's choline clinical trial on children 2-5 years of age were received in October 2020; cytokine assays and pre-processing of the data were completed by early 2021. However, in consultation with Dr. Wozniak, data analysis was put on hold. While data collection was excellent in the clinical trial overall, many children do not have the full set of blood samples (baseline, 6 months, 9 months) over the course of the trial. With the planned re-initiation of the clinical trial it was suggested that we delay data analysis in order to add samples to the study. However, while the trial has now been re-initiated, the blood draw was temporarily removed to reduce contact with participants and reassure parents that their children will be safe despite the ongoing pandemic. With uncertainty whether the blood draw can resume any time soon, we will now be working with Dr. Wozniak to find a way to do meaningful data analyses in the face of missing samples, including relating the cytokine data obtained to the rich data on eating behavior, changes in body weight, and health outcomes that are part of the trial.

Aim 2. Extend our assessment of the immune system in individuals with FASD into adulthood (Adult Health Study in collaboration with Drs. Claire Coles and Therese Grant).

No new recruitments were made since June 2021. To date we have recruited and tested 72/120 adults, including 18 with FAS, 28 with ARND, and 26 unexposed (mean age 37.5 yr for alcohol-exposed, 32 yr for unexposed).

Preliminary analyses to date indicate that groups did not differ in height, weight, blood pressure or heart rate. However, differences were observed in physical and mental health issues. Individuals with FASD had more days when they felt their health was not good, as well as more problems with their eyes, hearing, and teeth, more childhood heart problems, a higher incidence of asthma, epilepsy, kidney disease, diabetes, and thyroid problems, and higher levels of ADHD, depressive disorders, and psychotic disorder/schizophrenia. More of these individuals also experienced more than 2 adverse childhood experiences (ACEs) compared to their unexposed counterparts. As well, adults with FASD had higher white blood cell counts than unexposed adults, suggesting activation of the immune system. Finally, analysis of preclinical symptoms of autoimmune disorders (eg., skin problems [rashes, bruising], unexplained lack of energy, persistent fever or night sweats, dry mouth, alopecia, and pain or tenderness throughout the body) from our cohort and comparable data collected by Drs. Coles and Grant revealed that for the Vancouver and Seattle cohorts, adults with FASD had a significantly higher autoimmune score than their unexposed counterparts, whereas there were no differences between groups in the Atlanta cohort. Importantly, recruitment and testing of adults has resumed in both Atlanta and Seattle, and we will receive additional blood samples from both sites in early December for analysis. This will significantly enhance our database and allow for additional analysis and manuscript development over the next 6 months.

COVID-19 Study. Participants in the Vancouver cohort of the Adult Health Study were invited to participate by phone in a COVID-related study (January – May 2021) to assess the impact of the pandemic on mental health and stress levels,. Participants repeated the same battery of mental health and stress questionnaires as in the Adult Health Study, and also completed a set of COVID-related questionnaires (from the NIH repository of COVID-19 research tools). Preliminary results indicate that while depression scores increased for both adults with FASD and unexposed adults, anxiety scores increased to a greater extent in those with FASD. By comparison, pandemic stress levels were not different between adults with FASD and unexposed adults. Analyses of the COVID-related questionnaires are ongoing. Together, the data support the possibility that the psychological impact of the COVID-19 pandemic may be greater in vulnerable individuals, such as those with FASD, in some areas but similar to that in unexposed individuals in other areas. Importantly, recovery from the negative impacts of the pandemic should include plans and supports for addressing mental health concerns for adults with FASD. A manuscript based on these data is in preparation (Concept Proposal submitted).

Outreach to new collaborators. To compensate, at least partially, for delays faced by us and our collaborators due to COVID-19, we have established several new collaborations in order to expand our recruiting efforts:
a) Dr. Natasha Reid, University of Queensland (UQ), Australia*.* In her ongoing clinical study, Dr. Reid is collecting blood samples from children with FASD and matched controls to explore the development of biomarkers that reflect alcohol exposure *in utero*. We will obtain blood samples (blood spots), as well as demographic and health information, and cognitive, language, and behavioral assessments on these children, enabling us to assess the relationship of plasma cytokine levels to health and functional outcomes. Ethics approval is now complete and work is ongoing. ***b) Dr. Catherine Lebel, University of Calgary.*** One of Dr. Lebel's projects is examining structural and functional brain alterations (MRI) related to prenatal alcohol exposure in children 2-18 years of age, to investigate how brain abnormalities are related to diagnosis, facial dysmorphology, mental health symptoms, and other exposures. We will obtain blood samples (blood spots) from these children for measurement of cytokines, and will have access to all of the other measures in her study. Ethics approval is now complete and work is ongoing. Together, these two new collaborative studies will complement the studies in Aim 1, increasing our participant numbers and serving as comparison groups for further insight into relationships between immune/inflammatory alterations and health and functional outcomes.
c) Dr. Kaitlyn McLachlan, University of Guelph, Canada. Dr. MacLachlan's current research centers around FASD and neurodevelopmental disability across the lifespan. Working with Dr. McLaclan, we will reach out to adults with FASD and appropriate unexposed adults who are part of her research studies to recruit them to our Adult Health Study. Testing and blood sample collection will be done through her laboratory at Guelph. This collaboration could help us reach our Aim 2 target numbers in a more timely manner and could also serve as a comparison group to provide further insight into environmental/demographic factors influencing adult health and functional outcomes.

3) Key Outcomes and Other Achievements.

While we have been delayed in completing data collection for Aims 1 and 2, we are now moving forward toward meeting our goals. We are excited about our work with Dr. Miranda's group on several sets of samples to understand whether combined analysis of two molecular markers from maternal plasma could serve as stronger/more accurate predictors of child neurodevelopmental trajectories, and from child samples could serve as stronger/more accurate biomarkers of PAE/FASD compared to either marker alone. Additional samples from the Atlanta and Seattle cohorts of our Adult Health Study will be sent to us next week and assays run in December/January; this will move our data analysis forward. We are also optimistic that our new collaborations will complement our ongoing studies in both Aims 1 and 2, increasing our participant numbers and serving as a comparison group to provide further insight into relationships between immune/inflammatory alterations and functional outcomes. Finally, we have two new publications that have bearing on the work of CIFASD: 1) Lussier et al., *Genes*, 2021. This study utilized our animal model of PAE to investigate the consequences of two prenatal insults, prenatal alcohol exposure (PAE) and food-related stress, on DNA methylation profiles of the rat brain during early development, with a focus on sex-concordant and sex-specific epigenome-wide DNA methylation patterns in prefrontal cortex. Beyond our findings on FASD, we also identified overlapping DNA methylation patterns in FASD and autism spectrum disorder (ASD), which have relevance for the work of CIFASD. Despite differences in the core phenotypic characteristics of FASD and ASD, these neurodevelopmental disorders share several phenotypic characteristics, which are discussed. We also discuss reports of co-morbidity between PAE/FASD and ASD or autism-like symptoms, identify possible common pathways underlying ASD and FASD, and discuss possible links between the epigenomic mechanisms that may underlie these two disorders. 2) Lussier, Bodnar, Weinberg, *Frontiers in Neuroscience*, 2021. In this review paper, following an overview of the impact of PAE on immune function and epigenetic patterns, we discuss the potential role for epigenetic mechanisms in reprogramming of immune function and the consequences for health and development. We highlight a range of both clinical and animal studies to provide insights into the array of immune genes impacted by alcohol-related epigenetic reprogramming. Finally, we discuss potential consequences of alcohol-related reprogramming of immune/neuroimmune functions and their effects on the increased susceptibility to mental health disorders. Overall, the collective findings from animal models and clinical studies highlight a compelling relationship between the immune system and epigenetic pathways. These findings have important implications for our understanding of the biological mechanisms underlying the long-term and multisystem effects of PAE, laying the groundwork for possible novel interventions and therapeutic strategies to treat individuals prenatally exposed to alcohol.

Additional Questions

Final Year. What do you plan to do between now and May 2022* to accomplish your CIFASD4 goals and objectives? *For those with UH2s funded in 2021, what are your goals for year two of your support?

We will move forward towards completion of our Specific aims: We will complete and submit our papers on a) associations between maternal and child Immune milieus in alcohol-dependent and alcohol-independent neurodevelopmental delay, and b) on the impact of COVID-19 on adults with FASD. We will also focus on data analysis and development of manuscripts on the samples we have in hand: We will work with Dr. Miranda and team on combined analysis of two molecular markers (cytokines and miRNAs) in mother and child samples from the Ukraine cohort and child samples from the San Diego FASD Research Subject Pool; we will run cytokine assays on plasma samples from the Adult Health Study and work with Drs. Coles and Grant on data analysis, relating cytokine levels to cognitive and functional outcome measures; we will work with our new collaborators to extend our recruiting efforts to additional cohorts of children that will complement the studies in Aim 1, and to adults for participation in our Adult Health Study to meet our target n=120. Data analysis and manuscript development will be ongoing.

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 *Dr. 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. To date, two publications and one manuscript in preparation. As well, additional aliquots of plasma from blood samples taken from children and mother/child pairs in Dr. Chambers' new Ukraine birth cohort have been received and are being analyzed in parallel by Drs. Weinberg (cytokines) and Miranda (miRNA) to assess whether the use of multiple molecular markers can provide more sensitive predictors of child developmental trajectory and biomarkers of child risk/resilience than either cytokines or miRNAs alone.

2) Collaboration with *Drs. 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. Aliquots of plasma from 33 child samples have been received and again, will be analyzed in parallel by us and Miranda's group to assess whether the use of multiple molecular markers can provide more sensitive predictors/indicators than a single marker alone.
3) Dysmorphology Core. Three members 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 multiple subjects in our Adult Health Study via telemedicine consultation with Ken.
4) Collaboration with *Dr. Jeff Wozniak* on his choline clinical trial. Plasma samples from children with FASD receiving either choline or placebo have been received, and we will work with Dr. Wozniak on the analysis beginning in 2022.
5) Collaboration with *Drs. 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. Additional samples from both Atlanta and Seattle will be shipped to us next week, and assays will be run in December/January. Data analysis and manuscript preparation will be ongoing in 2022.
6) Weinberg has been providing 2D facial pictures of adults with FASD and controls to *Dr. Mike Suttie* for analysis under his U01. This will now be discontinued as Mike has indicated that these 2D photos are not useful for his analyses.
7) Interaction with *Drs. 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).

Administrative Supplements. For this reporting period (June 2021 to present), please list any Administrative Supplement associated with this award and a sentence or two about what it entails and its current status.

None

Publications/Abstracts

Publications [Accepted & In Press]

Lussier, A.A., Bodnar, T.S., Moksa, M., Hirst, M., Kobor, M.S., Weinberg, J. Prenatal adversity alters the epigenetic profile of the prefrontal cortex: Sexually dimorphic effects of prenatal alcohol exposure and food-related stress. *Genes 2021, 12, 1773. https://doi.org/10.3390/genes12111773*

Lussier, A.A., Bodnar, T.S., Weinberg J. Intersection of epigenetic and immune alterations: Implications for Fetal Alcohol Spectrum Disorder and mental health. Frontiers in Neuroscience (Specialty Section: Neurodevelopment). (Provisionally accepted pending final quality checks)

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]

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). *Concept Proposal 90.*

Bodnar, TS., Holman, PH, Chao, A, Ellis, L, Loock, C, Oberlander, T, Raineki, C, Weinberg, J, and the CIFASD. Impact of the COVID-19 pandemic on mental health and stress levels in adults with Fetal Alcohol Spectrum Disorders (FASD) (in preparation).

Poster Abstracts and Presentations

Holman, PJ. Effects of prenatal alcohol exposure on social behaviour neurobiology. Psychology Department Colloquium/BrewTap Series, Brock University, St Catharine's, ON, November 3, 2021.

Bodnar, TS., Holman, PH, Chao, A, Ellis, L, Loock, C, Oberlander, T, Raineki, C, Weinberg, J, and the Collaborative Initiative on Fetal Alcohol Spectrum Disorders (CIFASD). Impact of the COVID-19 pandemic on mental health and stress levels in adults with Fetal Alcohol Spectrum Disorders (FASD). FASDSG, June 16, 2021

Bodnar, T.S. [Symposium Organizer], Boschen, K.E., Przybysz, K.R., Raineki, C., Reid, N. Beyond prenatal alcohol exposure: Genes, environment, and diagnostic schemes as factors mediating outcomes. *Alcohol Clin Exp Res* 45:64A, 2021.

Raineki, C., Bodnar, T.S., Holman, P.J., Weinberg J. From animal models to humans: Role of early-life adversity on emotional regulation and immune function following prenatal alcohol exposure. In Symposium: Beyond prenatal alcohol exposure: Genes, environment, and diagnostic schemes as factors mediating outcomes. *Alcohol Clin Exp Res* 45:64A-65A, 2021

Coles, C.D., Grant, T., Weinberg, J., Kable, J.A., Radin, S., Smith-Stewart, T., Perez, A., and the CIFASD. Alcohol affected adults' self-reported health and mental health status: Preliminary evidence of long-term effects from a cross-site study. In Symposium: Health outcomes in adults with Fetal Alcohol Spectrum Disorders: A fetal basis to adult disease. *Alcohol Clin Exp Res* 45:69A, 2021

Miranda, R. and Dunty, B. [Organizers], Weinberg, J. [Moderator and Discussant]. Synposium: Health outcomes in adults with Fetal Alcohol Spectrum Disorders: A fetal basis to adult disease. *Alcohol Clin Exp Res* 45:68A, 2021.

Bodnar, T.S., Oberlander, t., Weinberg, J. Long-lasting changes in health and immune outcomes in adults with Fetal Alcohol Spectrum Disorder. In Symposium: Early-life adversity, inflammatory burden, and vulnerability: A translational approach. The Virtual 53rd Annual Meeting of the International Society for Developmental Psychobiology. October 21-23, 2020. Dev Psychobiol 62:S9, 2020.

Raineki, C., Bodnar, T., Holman, P., Weinberg, J. Assessing the impact of early-life adversity on emotionality and neuroimmune function in animals prenatally exposed to alcohol. In Symposium: Early-life adversity, inflammatory burden, and vulnerability: A translational approach. The Virtual 53rd Annual Meeting of the International Society for Developmental Psychobiology. October 21-23, 2020. Dev Psychobiol 62:S47, 2020.

Ranieri, C., Translational approaches for investigating how the early environment shapes neurodevelopmental trajectories and long-term health. *Concordia University, Department of Psychology* (Webinar due to COVID-19), July 16, 2020.

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 (withdrawn)

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. Alcohol Clin Exp Res 44:76A, 2020. CANCELLED

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

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.

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

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

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

Bodnar, T.S., Holman, P.J., Raineki, C., Weinberg, J. 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, 2019.

Raineki, C, Holman, PJ, Bodnar, TS, Weinberg, J. The impact of early-life adversity on emotional regulation and immune system function is mediated by adverse prenatal environment. In Symposium: 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, 2019.

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. Prenatal alcohol exposure disrupts the immune milieu: Impacts over the life course. In Symposium (Weinberg, Organizer and Chair): Neuroimmune dysfunction and health outcomes following prenatal alcohol exposure: Complementary cross-center perspectives. 42nd Annual Scientific Meeting of the Research Society on Alcoholism. Minneapolis, MN, June 22-26, 2019. Alcohol Clin Exp Res 43:261A, 2019.

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

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

Raineki, C., Holman, P.J., Bodnar, T.S., Weinberg, J. 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, 2019.

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. 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, 2018. Alcohol Clin Exp Res 42:46A, 2018

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. Childhood cytokine profiles are altered by -prenatal alcohol exposure: Risk vs. resilience signatures. Dev Psychobiol 60:10, 2018

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. 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, 2018.

Bodnar, T.S. Raineki, C., & Bandoli, G. 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, 2018.

Coles, C.D., Grant, T., & Weinberg, J. 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, 2018.

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

Weinberg, J. 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: Jeffrey R. Wozniak
Institution: University of Minnesota
CIFASD4 Project Title: Multi-modal connectivity methods for the validation of Fetal Alcohol Spectrum Disorder diagnostic criteria
Grant Number: U01 AA026102

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.

Accomplishments

1) Major Activities.

- Final number of participants enrolled as of November, 2021 is 101 (target was 90, but we exceeded the target to account for occasional MRI quality problems and to provide a larger pool of 15-month participants despite any loss to follow-up for that portion of the study)
- Number of participants who completed MRIs, 3D facial images, and neurocognitive testing is 101.
- Number of participants who completed second MRIs and neurocognitive testing at 15-months post enrollment = 77 (target was 60, but we exceeded the target to account for occasional MRI quality problems).
- Structural MRI data continue to be analyzed. Segmented volumetric data for most participants have been shared with other CIFASD investigators via Indiana University's Central Repository.
- Thus far, we have also used 561 brain images of typically developing children from the Human Connectome Project (development study) as a "normative" backdrop against which we are comparing our group with FASD for subcortical volumes. We have published one paper so far using this approach (Roediger et al., 2020, see below).
- Acquisition of 3D facial images for Dr. Michael Suttie's project: 101.
- Blood samples obtained from Dr. Wozniak's R01 project (R01AA024123-01) provided to collaborator Dr. Joanne Weinberg's CIFASD project on 10/8/2020; Provided thus far = 67 samples across 35 unique participants.
- Dr. Kenneth Jones has conducted dysmorphology exams on 70 children and adolescents enrolled in CIFSD. All of these participants also provided MRI and neurocognitive data.
- Salivary DNA and 2D photographs were collected from CIFASD participants and shared with Dr. Tatiana Foroud and Leah Wetherill at Indiana University. All of these participants also provided dysmorphology data, MRI, and neurocognitive data
- Neurocognitive and behavioral data were collected and shared in a joint database with Dr. Sarah Mattson at SDSU for her Decision Tree diagnostics project. Several papers have been published by Dr. Mattson's group on this work.
- Facilitating the use of neurocognitive data and neuroimaging data to Susan Smith's UH2 project on the SLC44A1 single nucleotide polymorphism.

2) Specific Objectives.

- Year 5 (6/1/2021 – 5/31/2022): We are using this time to analyze data and prepare manuscripts on the results. We have several sets of analyses underway including longitudinal DTI analyses (tractography) and longitudinal cortical structural analyses.

3) Significant Results.

- We have published several papers examining aspects of brain anomalies in our CIFASD population (see below) and are currently working on the longitudinal data.

- Challenges: We conducted an analysis of cortical myelin status, comparing those with PAE to controls, and found no differences. We attempted to publish these results in three different journals and were unsuccessful. This is very unusual and we believe it that the "negative" finding may be hindering the publication of the results. We are currently waiting a revised analytic pipeline to re-process the myelin data. With this technique, we may find a result or may have a stronger, more justifiable case for publishing the negative finding (greater certainty in the methodology).

4) Key Outcomes and Other Achievements.

We are finished with recruitment and with follow-up for all participants. We exceeded our targets for recruitment and for follow-up. All data are collected and we are in the process of conducting analyses and writing up manuscripts. We are also planning and preparing for a potential CIFASD-5 project, which will involve a neuromodulation intervention for children and adolescents with PAE.

Additional Questions

Final Year. What do you plan to do between now and May 2022* to accomplish your CIFASD4 goals and objectives? *For those with UH2s funded in 2021, what are your goals for year two of your support?

During this period, we plan to continue working on analyses and on preparation of manuscripts for publication. We also plan to prepare for a potential CIFASD-5 which would include an intervention study if the overall project is funded.

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

Our project provides data to Dr. Mattson's neurocognitive project and we are conducting shared analyses of the data. We provide 3D facial images to Dr. Suttie's project. We have provided DNA samples for genetic analyses and participant referrals to the Indiana University project (Wetherill & Foroud). We have provided blood samples to Dr. Weinberg's project on immune function. We have provided participants for Dr. Petrenko's focus groups and other application development needs. We have collaborated with the Dysmorphology core's projects (Jones and Del Campo). We have supplied blood samples to Susan Smith's genetics project.

Administrative Supplements. For this reporting period (June 2021 to present), please list any Administrative Supplement associated with this award and a sentence or two about what it entails and its current status.

None

Publications/Abstracts

Publications [Accepted & In Press]

1. Bernes, G, Courchesne, N., Hyland, M., Villodas, M., Coles, C., Kable, J., May, P., Kalberg, W., Sowell, E., **Wozniak, J.R.**, Jones, K., Riley, E., & Mattson, S. (in press). Development and Validation of a Postnatal Risk Score that Identifies Children with Prenatal Alcohol Exposure. *Alcoholism: Clinical and Experimental Research*.
2. 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.** (2021). Social Behaviors and Gray Matter Volumes of Brain Areas Supporting Social Cognition in Children and Adolescents with Prenatal Alcohol Exposure. *Brain Research; 2021 Feb 20;147388. doi: 10.1016/j.brainres.2021.147388.* PMID: 33621483. PMCID: PMC8377082.

3. Smith, S.M., Virdee, M.S., Eckerle, J.K., Sandness, K.E., Georgieff, M.K., Boys, C.J., Zeisel, S. H., & **Wozniak, J.R.** (2021). Polymorphisms in SLC44A1 predict which children diagnosed with FASD gain cognitive benefit from oral choline supplementation. *American Journal of Clinical Nutrition. doi: 10.1093/ajcn/nqab081. PMID:33876196; PMCID: PMC8326038.*

4. Rockhold, M.N., Krueger, A.M., de Water, E., Lindgren, C.W., Sandness, K.E., Eckerle, J.K., Schumacher, M.J., Fink, B.A., Boys, C.J., Carlson. S.M., Fuglestad, A.J., Mattson, S.N., Jones, K.L., Riley, E.P., CIFASD, and **Wozniak, J.R.** (2021). Executive and social functioning across development in children and adolescents with prenatal alcohol exposure. *Alcoholism: Clinical and Experimental Research*, 45(2), 457-469. doi: 10.1111/acer.14538. PMID: 33349933. PMCID: PMC7887051.

5. 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.** (2021). Hippocampal subfield abnormalities and memory functioning in children with fetal alcohol spectrum disorders. *Neurotoxicology & Teratology, 83, 1-8; dio:10.1016/j.ntt.2020.106944*. PMID:33232797. PMCID: PMC7855420.

6. 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; DOI: 10/1111/acer.14390;* PMID: 32524616; PMCID: PMC784415.

7. 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), https://doi.org/10.3389/fnhum.2020.00085*; PMID: 32322193; PMCID: PMC7156853.

8. **Wozniak, J.R.**, Riley, E.P., & Charness, M.E. (2019). Clinical Presentation, Diagnosis, and Management of Fetal Alcohol Spectrum Disorder. *The Lancet-Neurology,S1474-4422(19)30150-4*. PMID: 31160204; PMCID: PMC6995665.

9. 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;* DOI: 10.1111/acer.14036. PMID: 30908651; PMCID: PMC6551300

10. Sarkar, D., Gangisetty, O., **Wozniak, J.R.**, Eckerle, J., Georgieff, M., Foroud, T., Wetherill, L., Wertlecki, W., Chambers, C., Riley, E., Zymak-Kakutnya, N., Yevtushok, L. (2019). Persistent changes in stress-regulatory genes in pregnant woman or a child with prenatal alcohol exposure. *Alcoholism: Clinical and Experimental Research; 43(9):1887-1897;* DOI: 10.1111/acer.14148. PMID: 31329297; PMCID:PMC6722014

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

12. 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;* PMID: 29935097; PMCID: PMC6120799

13. 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. PMID: 29486453; PMCID: PMC5949095

14. Suttie, M., Wetherill, L., Jacobson, S.W., Jacobson, J.L., Hoyme E., Sowell, E.R., Coles, S., **Wozniak, J.R.**, Riley, E.P., Jones, K.L., Foroud, T., Hammond, P., and the CIFASD (2017). Facial curvature detects and explicates ethnic differences in effects of prenatal alcohol exposure. *ACER, 8: 1471-1483; DOI: 10.1111.acer.13429. PMID: 28608920; PMCID: PMC5563255.*

15. 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; doi: 10.1007/s11682-017-9739-2. PMID:28656347; PMCID:PMC5745322.*

16. Uban, K.A., Herting, M.M., **Wozniak, J.R.**, Sowell, E.R., and CIFASD. (2017). Sex differences in associations between white matter microstructure and gonadal hormones in children and adolescents with prenatal alcohol exposure. *Psychoneuroendocrinology, 83, 111-121. DOI: 10.1016/j.psyneuen.2017.05.019. PMCID: PMC5877456*.

17. Hendrickson, T.J., Mueller, B.A., Sowell, E.R., Mattson, S.N., Coles, C.D., Kable, J.A., Jones, K.L., Boys, C.J., Lim, K.O., Riley, E.P., & **Wozniak, J.R.** (2017). Cortical gyrification is abnormal in children with Prenatal Alcohol Exposure. *Neuroimage: Clinical. 15, 391-400; doi.org/10.1016.j.nicl.2017.05.015.* PMCID: PMC5447653.

Publications [In Preparation & Submitted]

None under review at the moment.

Poster Abstracts and Presentations

1. 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 presented at the International Neuropsychological Society Conference, San Diego, CA.
2. 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 presented at the International Neuropsychological Society Conference, San Diego, CA.
3. 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.
4. 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.
5. 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.
6. 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.

7. Wetherill, L., Nudelman, K., Schwantes-An, T.H., Boes, J., Parnell, S.E., Abreu, M., Coles, C., Jones, K., Kable, J., Kan, E., Lai, D., Sowell, E.P., **Wozniak, J.R.**, Riley, E.P., Mattson, S.N., & the CIFASD. (2019, June). Long-term follow-up of a randomized controlled trial of choline supplementation in children with fetal alcohol spectrum disorders (FASD) (poster presented at the 42nd Annual meeting of the Research Society on Alcoholism, Minneapolis, Minnesota).
8. 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 at the annual meeting of the International Neuropsychological Society, Washington D.C.

Principal Investigators: Annika Montag and Christie Austin
Institutions: UCSD & Mount Sinai
CIFASD4 Project Title: Development of biomarkers in deciduous teeth of children with FASD that predict neurobehavioral performance
Grant Number: UH2 AA029062

Specific Aims

Aim 1. Determine the sensitivity and specificity of direct and indirect biomarkers of PAE in deciduous teeth of 25 children with FASD and 25 children with known absence of PAE.

Aim 2. Assess associations among magnitude and gestational timing of PAE identified in the teeth of 25 children with FASD and 25 children with known absence of PAE and neurobehavioral deficits.

Aim 3. Explore the interaction between PAE and exposures to neurotoxic and nutritive metals during prenatal and early life as measured in the deciduous teeth of 25 children with FASD and 25 children with known absence of PAE.

Accomplishments

1) Major Activities. Created materials and obtained UCSD IRB approvals. Put material transfer agreement and contracts in place between UCSD and Mount Sinai. Trained staff and purchased supplies. Drs. Mattson and Wozniak disseminated our flyer and "permission to contact" information to their eligible cohort participants.

2) Specific Objectives. In addition to accomplishing the "major activities" as described above, we had hoped to have recruited participants at this point. We were delayed by the time required to put contracts and material transfer agreements in place between UCSD and Mount Sinai. Nonetheless, we expect to complete our aims within the original timeframe.

3) Significant Results. No biomarker results have been obtained to date

4) Key Outcomes and Other Achievements. NA

Additional Questions

Final Year. What do you plan to do between now and May 2022* to accomplish your CIFASD4 goals and objectives? *For those with UH2s funded in 2021, what are your goals for year two of your support?

Recruit participants and initiate analysis of samples

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

We interact with CIFASD projects led by Dr. Sarah Mattson and Dr. Jeffrey Wozniak. We are recruiting participants from their cohorts and will investigate associations among the dental biomarkers we identify and previously collected neurobehavioral data for the same individuals. Contact has been initiated with a few participants in Mattson/Wozniak cohorts.

Administrative Supplements. For this reporting period (June 2021 to present), please list any Administrative Supplement associated with this award and a sentence or two about what it entails and its current status.

NA

Publications/Abstracts

Publications [Accepted & In Press]

NA

Publications [In Preparation & Submitted]

We are preparing a manuscript of our proof-of-concept preliminary study.

Poster Abstracts and Presentations

A poster and video poster were presented at the RSA 2021 conference. Title: *Can naturally shed baby teeth from young children be used to map their history of prenatal alcohol exposure?* Abstract #656

Principal Investigators: Christie Petrenko and Cristiano Tapparello
Institution: University of Rochester
CIFASD4 Project Title: Mobile Health Tools to Promote Health in Adults with FASD
Grant Number: UH2 AA029050

Specific Aims

Aim 1. Development of "My Health Coach:" Using a just-in-time adaptive intervention design, develop a novel mHealth app to increase health literacy, social support, self-management, and self-advocacy abilities of adults with FASD. These targeted outcomes will promote health and well-being.
Aim 1a. Identify and refine functionalities required to efficiently address the health management needs of adults with FASD through focus group and survey methods.
Aim 1b. Develop an iOS prototype of the mHealth app for subsequent testing.

Aim 2. Feasibility Study: Using qualitative and quantitative methodologies, assess the feasibility and user satisfaction and experiences with the innovative My Health Coach application built in Aim 1, involving a diverse group of 40 adults with FASD.

Accomplishments

Our team is making excellent progress on our stated goals. This progress is outlined by activity below.

Establish Infrastructure to Partner with our Community Advisory Board of Adult Leaders with FASD

We have established a strong partnership between our research team members at the University of Rochester and our community advisory board (CAB) of adult leaders with FASD. Our CAB includes the following members: Myles Himmelreich, Emily Hargrove, Katrina Griffin, Maggie May, Anique Lutke, CJ Lutke, and Midori Harth. We value the motto "Nothing about us, without us." We view our CAB members as integral members of our team and strive to ensure they experience full inclusion in our research project.

To make our CAB most successful, at the start of the project we conducted a literature review and consulted with experts within our CTSI to learn known CAB best practices. We reviewed these best practices with our CAB and obtained their input. From that, we have implemented strategies such as a project road map to inform the team on accomplishments, current tasks, and next steps of the project. We also co-created a roles and responsibilities agreement with our CAB and provide pre- and post-meeting materials. Lastly, we set aside time each meeting to share and celebrate successes of our team in both professional and personal lives.

As of 12/1/2021, we have met with our CAB 14 times. In addition to initial infrastructure and group building, our meetings have focused on identifying and refining app functionalities and interface design, preparing for presentations, and focus group planning. These are discussed in subsequent sections.

Identifying and Refining App Functionalities and Interface Design

Consistent with our systematic user-centered design approach to app development, we engaged our CAB to identify and refine key functionalities of the app. CAB members considered findings from their prior "Lay of the Land" surveys of over 400 of their peers and their own personal experiences in this process. Next, we began co-designing the app interface and considering how to implement functionalities in a simple and engaging format for users. Our research assistant Emily Speybroeck used Adobe XD to create and refine an interactive prototype that we reviewed and integrated CAB member feedback iteratively across meetings. CAB members had many insightful and practical suggestions, and this was a very productive and exciting process. See several screenshots at the end of this section.

Focus Group Planning & Recruitment

As indicated in Aim 1a, the next step in our systematic user-centered approach to app development and evaluation is to conduct focus groups with a broader range of adults with FASD. We had originally planned to conduct these focus groups as part of the International FASD Conference in Vancouver that was scheduled for April 2021. Unfortunately, due to the ongoing COVID-19 pandemic this conference was cancelled. As indicated in the original grant application, we planned to move to online focus groups in such a scenario. With CAB member input, we decided to move focus group data collection to Quarter 3 of Year 1 (vs. near the start of the grant) to allow us adequate time to fully flush out the app functionalities and interface design. CAB members advocated

that having concrete visual examples (such as in our interactive prototype) would elicit more useful feedback than engaging participants earlier in the design process.

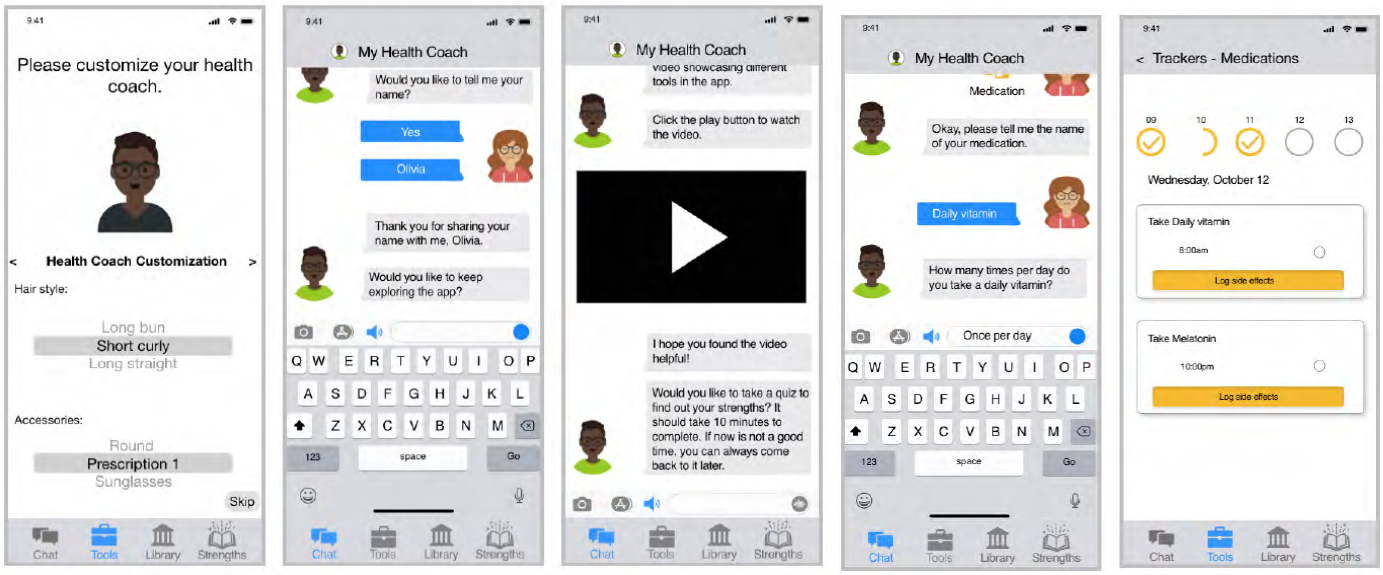
To that end, we submitted a modification to our IRB approval in September, 2021. We created recruitment materials and an online registration database in REDCap for interested participants to sign up for the study. Rolling recruitment began October 13. CAB members have been active in sharing information about the study in their social media networks. They report a high level of enthusiasm among members of these groups. We encountered an initial enrollment barrier soon after recruitment materials were distributed when the service within REDCap that provides shortened survey links experienced an outage for 9 days. We were able to quickly send out alternate sign-up options (e.g., longer link, research assistant email, QR code) and the shortened link issue has subsequently been resolved.

At recent CAB meetings we have refined the questioning route to be used in the focus groups and have role-played scenarios so CAB members feel comfortable leading focus group discussions and interacting with the research member paired with them in their Zoom breakout room. We are aiming to have our first focus group the first weekend in December.

Presentations at Scientific Conferences

We have co-presented our work on developing the My Health Coach app with our CAB members at two conferences to date: First, we presented a virtual poster at the 2021 FASD Study Group meeting in June. All research and CAB members contributed to the poster design and accompanying 5-minute video presentation. Next, we presented a 75min symposia at the 2021 ProofCon meeting through Proof Alliance. This included scientific and theoretical background of the My Health Coach app, presentation of CAB best practices, panel discussion with CAB members, and demonstration of the interactive app prototype with audience feedback. This session received some of the highest ratings across conference sessions.

Dr. Petrenko has also presented on the My Health Coach app, as part of mHealth interventions under development for FASD at several recent conferences, including the European Society for Biomedical Research on Alcohol conference (October 2021) and the Australasian Professional Society on Alcohol & other Drugs FASD Special Interest Group meeting (November 2021).



Additional Questions

Final Year. What do you plan to do between now and May 2022* to accomplish your CIFASD4 goals and objectives? *For those with UH2s funded in 2021, what are your goals for year two of your support?

Following completion of focus group data collection, all recordings will be transcribed verbatim and analyzed qualitatively. Preliminary findings will be presented to CAB members to aid in meaning making and refining the analytic framework.

As planned in Aim 1a, we will also develop an online survey to aid in eliciting further input from adults with FASD. This survey will include questions follow-up from common themes or decision points gathered in focus groups (e.g., selecting preferred app icon design, wording, inclusion of feature).

We will also begin programming the app using cross-platform toolkits and frameworks well known to our team. This will include determining decision points and responses for app features. Additional content will also be developed by our team (e.g., factsheets for Library, bank of message of day). As the programmed prototype evolves, we will do internal testing within our team and CAB members. In the second half of year 2, we will initiate a pilot RCT as planned, including enrolling 40 adult participants with FASD to test the app.

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 across the lifespan. Within CIFASD-5, our project is both a recipient and referral source for participants. To date, other CIFASD PIs recently sent out recruitment information for our focus group study. In the future, we may be able to aid other projects in recruitment/referral.

Administrative Supplements. For this reporting period (June 2021 to present), please list any Administrative Supplement associated with this award and a sentence or two about what it entails and its current status.

n/a

Publications/Abstracts

Publications [Accepted & In Press] N/A

Publications [In Preparation & Submitted] N/A

Poster Abstracts and Presentations

Petrenko, C.L.M., Cole, L., & Kautz-Turnbull, C. (2021). Leveraging technology to increase access to diagnosis and fetal alcohol spectrum disorder (FASD)-informed care across the lifespan. Australasian Professional Society on Alcohol and Other Drugs FASD Special Interest Group meeting. November 7, 2021.

Petrenko, C.L.M., Tapparello, C., Speybroeck, E., Griffin, K., Hargrove, E., Himmelreich, M., Lutke, C.J., Lutke, A., May, M., & Harth, M. (2021). "Nothing about us without us": Partnering with adults with FASD to develop mobile health tools for condition management. 2021 Proof Alliance Conference: ProofCon 2021: Empowered by the Proof., October 2021.

Petrenko, C. L. M. (2021). Development and evaluation of digital and mobile health interventions (eHealth) for FASD. Virtual Presentation in symposia "Diagnosis and Interventions in FASD: From Genes to eHealth." European Society for Biomedical Research on Alcohol, Timisoara, Romania, October 2021.

Speybroeck, E., Petrenko, C.L.M., Tapparello, C., Kautz-Turnbull, C., Demir, U., Griffin, K., Hargrove, E., Himmelreich, M., Lutke, C.J., Lutke, A., May, M., & Harth, M. (2021). "Nothing about us without us": Partnering with adults with FASD to develop mobile health tools for condition management. Virtual poster presentation at 2021 Fetal Alcohol Spectrum Disorders Study Group annual meeting, June 2021.

Principal Investigator: Susan Smith
Institution: University of North Carolina Nutrition Research Institute
CIFASD4 Project Title: Choline Polymorphisms in FASD
Grant Number: UH2 AA029056

Specific Aims

Aim 1. We hypothesize that, in the OMNI-Net choline intervention trial, the SLC44A1 minor variants rs3199966(G) and rs2771040(G) predict neurobehavioral performance and response to choline supplementation for the alcohol-exposed infants.

Aim 2. We hypothesize that, for individuals diagnosed with FASD within the Phase 2/3 CIFAD database, the SLC44A1 minor variants rs3199966(G) and rs2771040(G) will be associated with greater cognitive deficits compared with its major allele.

Aim 3. We hypothesize that the major and minor variants in rs3199966 and rs2771040 have distinct functional effects upon SLC44A1-mediated choline uptake and utilization.

Accomplishments

Major Activities & Specific Objectives

To date, data were only available for Aim 2. We received access to the CIFASD2/3 phenotypic data in April 2021. We requested the Genetics Core to impute the relevant SNPs associated with the target gene SLC44A1 for all 545 samples in Phase 2 and 3, and we received these data in June 2021. We have spent the subsequent time preparing these data for the association analysis. This began with the review of all the phenotypes (neurobehavior, dysmorphology) in those data. The data dictionary for Phase 3 was complete, whereas that for Phase 2 was incomplete, and both only partially overlapped and thus required interpretation before we could merge these for validation and analysis. We also found that individuals overlapped between these datasets, and this necessitated additional meetings to sort out who was who, and which data went with which individuals. It emerged that some individuals were duplicated in the data, some were seen multiple times, and these had to be identified and sorted within the datasets.

We also encountered complications with both the genetic and phenotype co-variate data files. Specifically, the relevant covariant – race and ethnicity – were missing from the dataset, and we needed these to calculate the eigen values for the genetics from the PCA. This required some discussion as there was initial confusion about the data needed to perform this, and was further complicated by data validation errors. Eventually we obtained these data and performed this analysis and verified that the genetic data did correspond with the samples, and thus we were able to implement genetic covariants in our model. With respect to the phenotype covariants, we did not possess a rigorous diagnostic category for these individuals; we were originally supplied with a heterogenous group that contained a broader range of diagnostic classes (ADHD, control, FAS, etc) and were unable to discern who was who. Again, another request was made and eventually we were provided with recruitedchildgroup. What we now await is a category that takes all diagnoses into consideration, so we can extract the relevant alcohol-exposed individuals, and we are still waiting to receive the demographic group class (Demgroupclass) so that we can perform that analysis.

We also invested significant effort in data cleansing, imputation, and validation, to prepare the phenotypic measurements for the analysis. During our data exploration, we identified inconsistencies within the data as they were being entered into the datasheets. For example, missing variables were inconsistently identified, some using NA, some using 9999, and others an empty cell. Additionally, data sheets presented raw data alongside calculations, which limited the ability to check the individual cells for accuracy and data validation. Confusing this were the duplicated exam arms, in which the same individuals appeared in both CIFASD 2 and 3, and these needed to be identified and either removed or consolidated, as we could not determine if they were redundant. For much of this, we lacked the knowledge necessary to resolve the problem, and this required much back and forth between us and the data repository.

With this completed, we then turned to data transformation so that it would be acceptable to enter the data model for the association analysis. We then went to test these in a data subset, focusing on SLC and the demographic data, to explore both covariates and potential endpoints of interest. Below is a sample of this analysis; however, this is incomplete because we are still awaiting the demgroupclass for accurate data interpretation. This is an example of the data we hope to produce, now that the data cleansing is largely complete and once the demgroupclass arrives from the repository. Our next steps will focus on the model development, identifying covariates and perhaps looking at datasubsets, but again this cannot commence without the

demgroupclass identities and a complete knowledge of covariates (genes, age, sex, IQ, ethnicity, possibly face, height, and other phenotypes).

In the meantime, we performed a very preliminary analysis, holding age of examination, sex, and identity by descent as co-variates, and comparing an association with palpebral fissure length, as it was a phenotype entry that required little cleansing. This involved 205 samples from Phase 3 that passed the identity by descent.

rs3199966	q=0.0253	Increased w/Minor Allele (G)	r= -6.237	76/205 carriers
rs2771040	q=0.0468	Increased w/Minor Allele (G)	r= -5.686	84/205 carriers

These data indicate, first, that the relevant SLC44A1 effect alleles have sufficient representation in the CIFASD2/3 population to provide sufficient power in our analyses, on the order of 37-41%. Second, these q-values are in the range of those obtained in our analysis of these SNPs against cognitive performance in the elicited imitation task (Smith et al. 2021), thus providing proof-of-principle that our approach should work in this cohort. Third, it identifies a tentative association between these SNPs and the palpebral fissure length, which is unexpected and may benefit from additional exploration.

Aim 1. We have been awaiting completion of the genomic sequencing of the OMNI-NET cohort. This is now complete. The contractor is now reviewing the clinical data and will release the sequence data once they have "cleared" it. We are told this will be completed after the winter break. At that point, we will request SNP identities within SLC44A1 and commence that analysis against relevant phenotypes.

Aim 3. We have obtained the relevant neuronal cell line and are designing the Cas9 target sequences for the targeted mutagenesis of the two SNP loci.

3) Significant Results. None yet.

4) Key Outcomes and Other Achievements. None yet.

Additional Questions

Goals for Year 2.

We will perform the association analysis within CIFASD2/3 between SLC44A1 effect alleles and cognitive outcomes in the alcohol-exposed population. We will request the relevant SNPs from the OMNI-NET analysis and conduct that parallel association analysis, following data cleansing of those phenotypes. We will complete construction of the neuronal cells harboring the relevant effect SNPs and test their impact on choline transport and fate.

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

Awaiting outcomes of the genetic association studies.

Administrative Supplements. For this reporting period (June 2021 to present), please list any Administrative Supplement associated with this award and a sentence or two about what it entails and its current status.

Not relevant.

Publications/Abstracts (please list from most recent first back to June 2017 - the start of CIFASD4)

None.