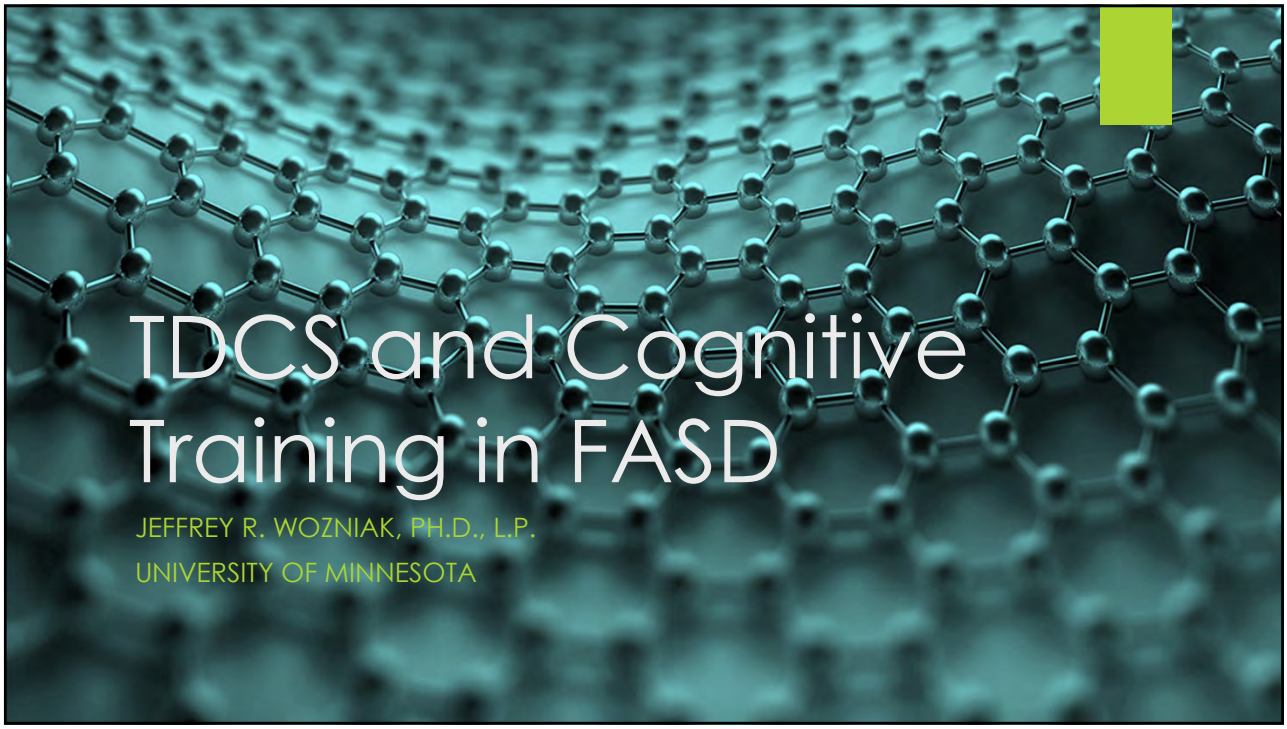


Specific Aims

Aim 1. Five sessions active tDCS (n=30) vs. sham tDCS (n=30) with CT (n=60; all participants). **Hyp1:** Active tDCS -> sustained attention (CPT) and parent-reported ADHD symptoms compared to sham tDCS over 5 sessions.

Aim 2. Quantify dose-response. **Hyp2:** With CT (n=60; all participants), 10 active tDCS sessions (n=30) -> sustained attention (CPT) and parent-reported ADHD symptoms compared to 5 active + 5 sham sessions (n=30).

Aim 3. Durability. **Hyp3:** Two months post-intervention, sustained attention (CPT) improvements vs. baseline performance will remain. **Hyp4:** Dosage will be related to durability (10>5)

Aim 4 (exploratory): fMRI -- brain network activity between baseline and 5 sessions (active tDCS vs. sham tDCS). **Hyp5:** 5 sessions of active tDCS (n=15) will enrich connectivity compared to 5 sessions of sham tDCS (n=15).

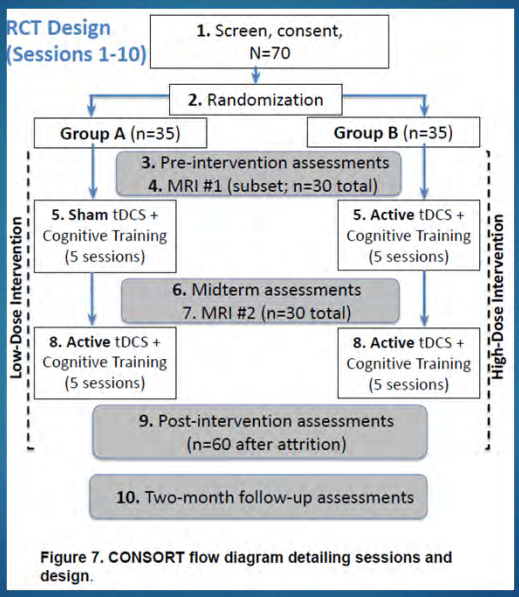


Figure 7. CONSORT flow diagram detailing sessions and design.

Progress

- IRB approval: 6/10/2022
- Project well on track

Progress

- First participant enrolled 11/10/2022
- 14 participants enrolled (20% of overall target)
  - Completed 127 sessions of testing, cognitive training, and neuromodulation
  - 25 MRI scans completed
  - 2 participants dropped
  - 9 follow-up (durability) visits
- Great response to our recruitment efforts

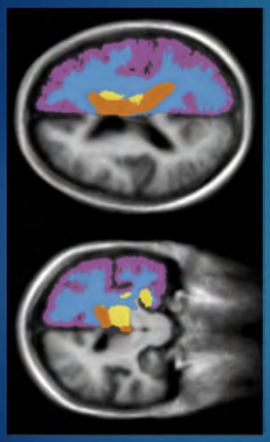
Interactions

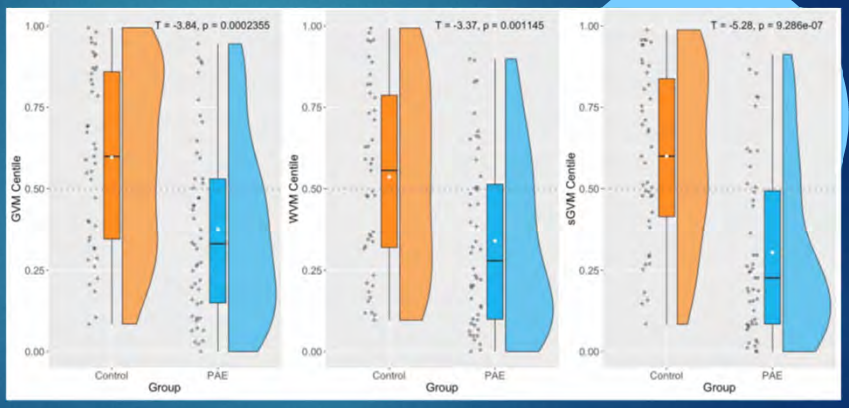
- **Miguel del Campo, UCSD** – Completed training of multiple personnel; Data collected on 14 participants; Awaiting database construction (will *enter data* or pass datasheets to UCSD)
- **Sarah Mattson, SDSU** – Participants being sent to BRAIN-online at conclusion of study
  - Spinoff project (Blake Gimbel: grant to study BRAIN-online and clinical neuropsych)
- **Mike Suttie, Oxford** – 14 participant images shared
  - Canfield system
- **Leah Wetherill, Indiana U.**
  - Data dictionary complete
  - GUIDs being generated for each participant
  - Upload tests completed
  - All participant data uploaded and current

Dissemination / Advocacy

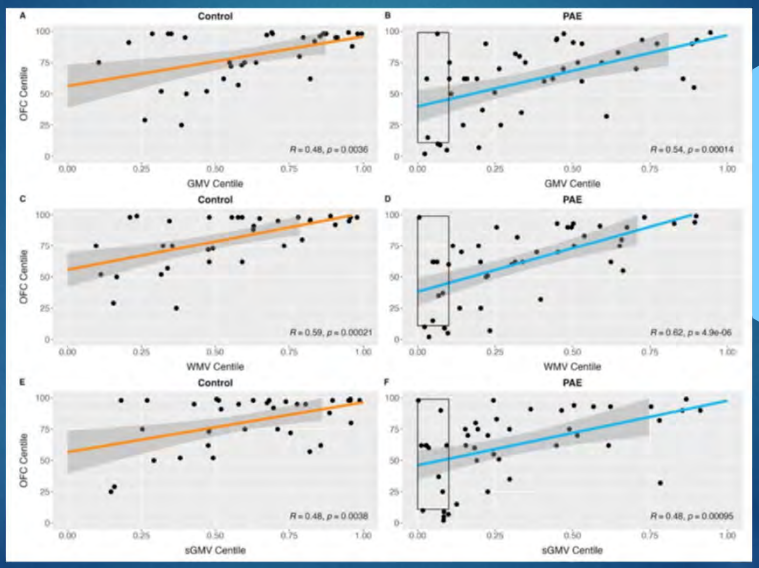
AMERSA (The Association for Multidisciplinary Education and Research in Substance use and Addiction)

Gimbel, B.A. Roediger, D.J., Ernst, A.M., Anthony, M.E., Mueller, B.A., deWater, E., Rockhold, M.N., CIFASD, & Wozniak, J.R. (in press). Normative MRI data increase the sensitivity to brain volume abnormalities in the classification of FASD. Journal of Pediatrics.

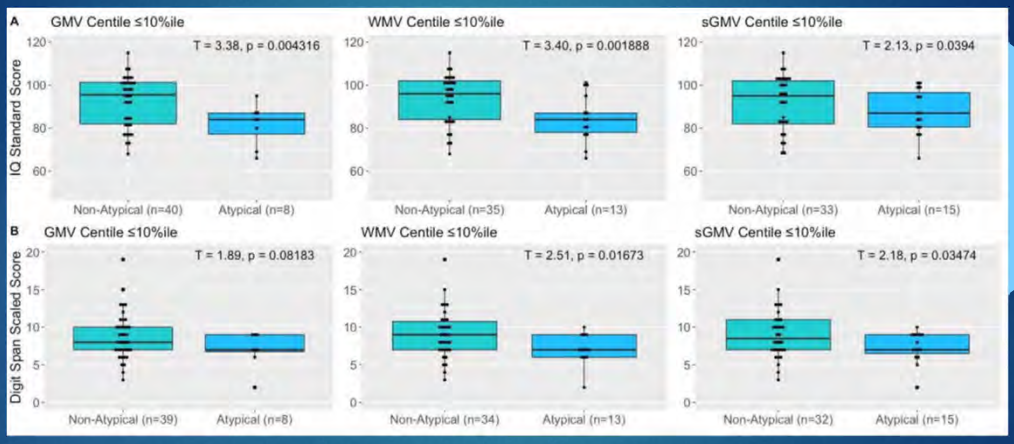




Gimbel, B.A. Roediger, D.J., Ernst, A.M., Anthony, M.E., Mueller, B.A., deWater, E., Rockhold, M.N., CIFASD, & Wozniak, J.R. (in press). Normative MRI data increase the sensitivity to brain volume abnormalities in the classification of FASD. Journal of Pediatrics.



Gimbel, B.A. Roediger, D.J., Ernst, A.M., Anthony, M.E., Mueller, B.A., deWater, E., Rockhold, M.N., CIFASD, & Wozniak, J.R. (in press). Normative MRI data increase the sensitivity to brain volume abnormalities in the classification of FASD. Journal of Pediatrics.



Gimbel, B.A. Roediger, D.J., Ernst, A.M., Anthony, M.E., Mueller, B.A., deWater, E., Rockhold, M.N., CIFASD, & Wozniak, J.R. (in press). Normative MRI data increase the sensitivity to brain volume abnormalities in the classification of FASD. Journal of Pediatrics.

	Sensitivity	Specificity	PPV	NPV	Accuracy
Height or weight ≤ 10%ile	10.42%	93.02%	62.50%	48.19%	49.45%
OFC ≤ 10%ile	11.11%	100.0%	100.0%	46.67%	50.0%
≥ 2 facial features	26.67%	94.29%	85.71%	50.0%	56.25%
3 facial features	6.67%	100.0%	100.0%	45.45%	47.50%
Low IQ (≤ 1.5 SD below mean)	18.75%	100.0%	100.0%	51.85%	56.67%
GMV or WMV or sGMV ≤ 10%ile	35.42%	95.35%	89.47%	56.94%	63.74%
OFC ≤ 10%ile or brain atypicality	36.96%	94.44%	89.47%	53.97%	62.20%

Additional papers from CIFASD4 year 2

Smith, S.M., Weathers, T.D., Virdee, M.S., Schwantes-An, T.H., Mattson, S.N., Coles, C., Kable, J., Sowell, E., **Wozniak, J.R.**, Wetherill, L., and the CIFASD. (in press). Polymorphisms in the choline transporter SLC44A1 are associated with reduced cognitive performance in both normotypic and prenatal alcohol-exposed children. *American Journal of Clinical Nutrition.*

Hyland, M., Courchesne, N., Bernes, G., **Wozniak, J.R.**, Jones, K.L., del Campo, M., Riley, E., & Mattson, S. (2023). Results of an FASD Screening Tool are Associated with Neuropsychological and Behavioral Measures. *Alcoholism: Clinical and Experimental Research, 47(8), 1560-1569. DOI:10.1111/acer15133*

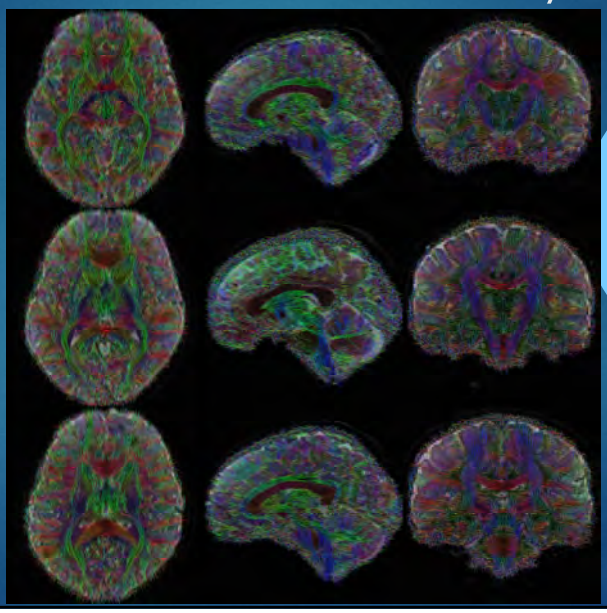
Gimbel, B.A., Roediger, D.J., Ernst, A.M., Anthony, M.E., deWater, E., Rockhold, M.N., Mueller, B.A., Mattson, S.N., Jones, K.L., Riley, E.P., Lim, K.O., CIFASD, & **Wozniak, J.R.** (2023). Atypical developmental trajectories of white matter microstructure in prenatal alcohol exposure: Preliminary evidence from neurite orientation dispersion and density imaging (NODDI). *Frontiers in Neuroscience, DOI: 10.3389/fnins.2023.1172010*

Gimbel, B.A., Roediger, D.J., Ernst, A.M., Anthony, M.E., deWater, E., Mueller, B.A., Rockhold, M.N., Schumacher, M.J., Mattson, S.N., Jones, K.L., Lim, K.O., CIFASD, & **Wozniak, J.R.** (2023). Delayed cortical thinning in children and adolescents with prenatal alcohol exposure. *Alcoholism: Clinical and Experimental Research, doi: 10.1111/acer.15096*

Mattson, S.N., Jones, K.L., Chockalingam, G., **Wozniak, J.R.**, Hyland, M.T., Courchesne-Krak, N.S., Del Campo, M., Riley, E.P., and the CIFASD. (2023). Validation of the FASD-Tree as a screening tool for fetal alcohol spectrum disorders. *Alcoholism: Clinical and Experimental Research*, 47(2), 263-272; DOI: 10.1111/acer.14987

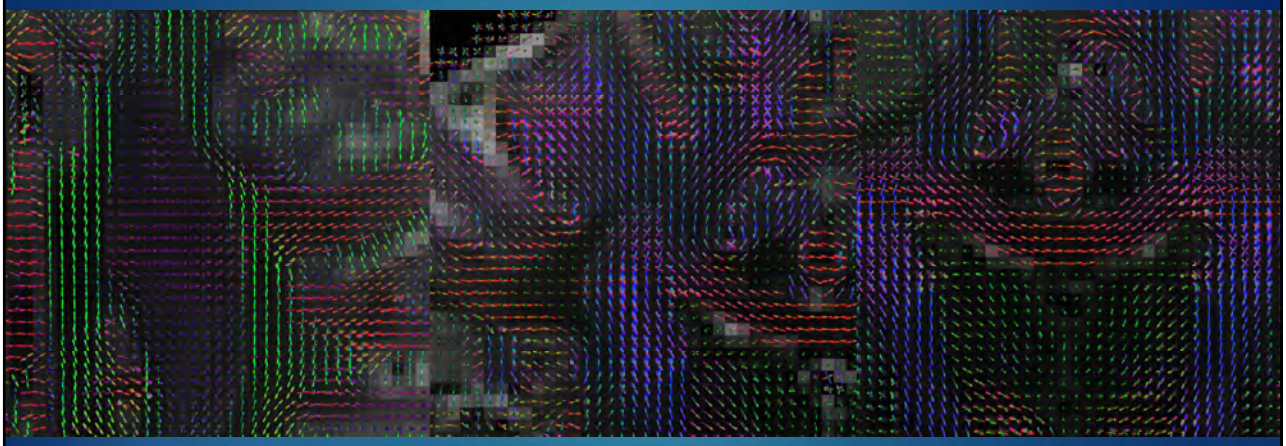
Bernes, G.A., Courschesne-Krak, N.S., Hyland, M.T., Villodas, M.T., Coles, C. D., Kable, J.A., May, P.A., Kalberg, W.O., Sowell, E. R., **Wozniak, J.R.**, Jones, K.L., Riley, E.P., Mattson, S.N., and the CIFASD. (2022). Development and validation of a postnatal risk score that identifies children with prenatal alcohol exposure. *Alcoholism: Clinical and Experimental Research, 46(1):52-65; DOI: 10.1111/acer.14987*

Ongoing structural connectivity work



- 11 year old
- Female
- ARND

Ongoing structural connectivity work



A Multisite Study of Prenatal Alcohol Exposure: Effects of Inflammation and Endocrine Dysfunction in Adulthood
NIH/NIAAA #: U01AA026108

Claire D. Coles, PhD
Joanne Weinberg, PhD
and
Susan Stoner, PhD
Tamara Bodnar, PhD
University of Calgary, Alberta
Charlis Raineki, PhD
Brock University, Ontario

Sites

- ❑ Emory University School of Medicine Atlanta, GA
- UBC, Vancouver, BC
- U Calgary, Alberta
- Brock University, Ontario
- University of Washington Seattle, Washington

1

Structural Changes

- MPI Change- Joanne Weinberg, PhD, will step down as MPI on January 1, 2024 and continue as a collaborator/advisor
- Tamara Bodner, PhD, University of Calgary, Alberta will become the new MPI and the subcontract will be transferred to Calgary.
- Major activities in recruitment and analysis of biological samples will be carried out in Calgary.
- Charlis Raineki, PhD, Brock University, Ontario will work closely with Dr. Bodner to carry out project goals.
- Decision on these changes were supported by NIAAA staff.

2

- USA Progress Checklist
  - IRB
  - Data Dictionary
  - Practice Data set
  - GUID Training
  - # of subjects, Current
  - # of Subjects, Projected by end of Year 2
  - Samples Shared/To share
  - Dysmorphology Training
  - 3-D Camera/Photographs

- **IRB**-sIRB obtained 9/22 for US site. Yearly renewal is not required for sIRBs.

Data Sharing

- **Data Dictionary**-Completed. Submitted to Indiana. Revised 12/8/23
- **Practice Data Set:** Submitted and amended. Corrections made and resubmitted. 12/14/2023

3

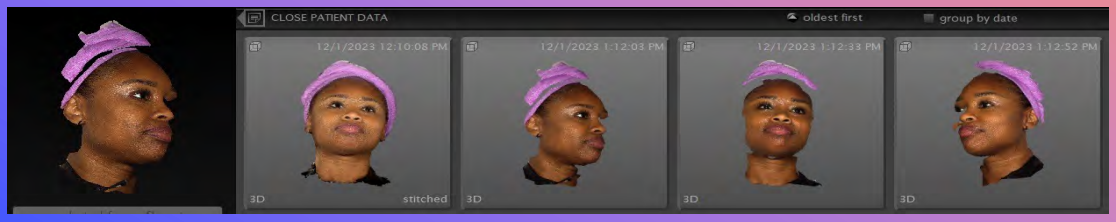
US Data Collection

- **Began DATA collection**-6/23.
- Anticipated final sample: 240 (120 from ATL/SEA each)
  - **Current total**: 51 (ATL and SEA) (21%)
  - **Anticipated by 3/24**: 88 (37%)
- **Samples stored for sharing**-same as # subjects.
- **Dysmorphology**- Initial Training completed 10/18; Others scheduled.

4

PROGRESS ON 3-D IMAGERY

- Working with Mike Suttie, Atlanta received the 3-D Camera and software. Seattle will receive another camera
- Trained staff on use of camera
- Began collecting photos in December 2023.



5

5

Data sets being analyzed

(Concept Proposals submitted to CIFADS)

- Health Status: Effects of Prenatal Exposure
  - Examining those who define themselves as Healthy and Unhealthy in the Tier 1 dataset and determining the physical problems they report to identify those most commonly associated with FASD. Significant effects of PAE on: **Hearing**, **Dental Health**, **Kidney/Urinary Function** when other variables controlled.
- Patterns of Dysmorphology in Adults with Prenatal Alcohol Exposure
  - In individuals examined in Tier 2, comparing the features usually associated with FASD in childhood to identify those that could be use in adult diagnosis.
- Prenatal exposure and Substance Use
  - It is frequently assumed that prenatal alcohol exposure will predict later substance use. This sample allowed us to examine this question.

6

Data Collection for Substance Use Study

- Drug Checklist
  - Current Drug use
  - Drug Use Ever

→ Alcohol, Tobacco, Marijuana, Cocaine, Methamphetamine, Opioids (heroin, prescription medications, other), Tranquillizers, Sedatives, Hallucinogens, Ecstasy, Inhalants, Other drugs

- Alcohol Specific
  - AUDIT Questionnaire
  - GGT-Blood test

→ Total AUDIT score, Cut Off Score, GGT levels, Thresholds

- Urine Screen for Current Drug Use

→ Benzodiazepine, Barbiturate, Cocaine, Marijuana Methamphetamines, Opiates, Methadone, Phencyclidine, Amphetamines

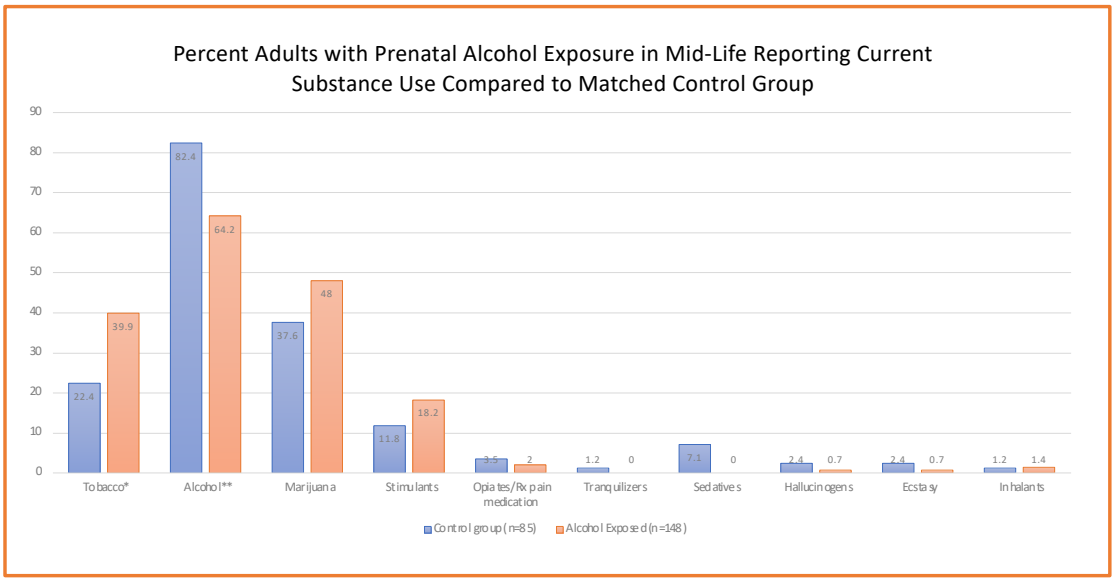
7

Demographics of Tier 2 Sample (N=233)

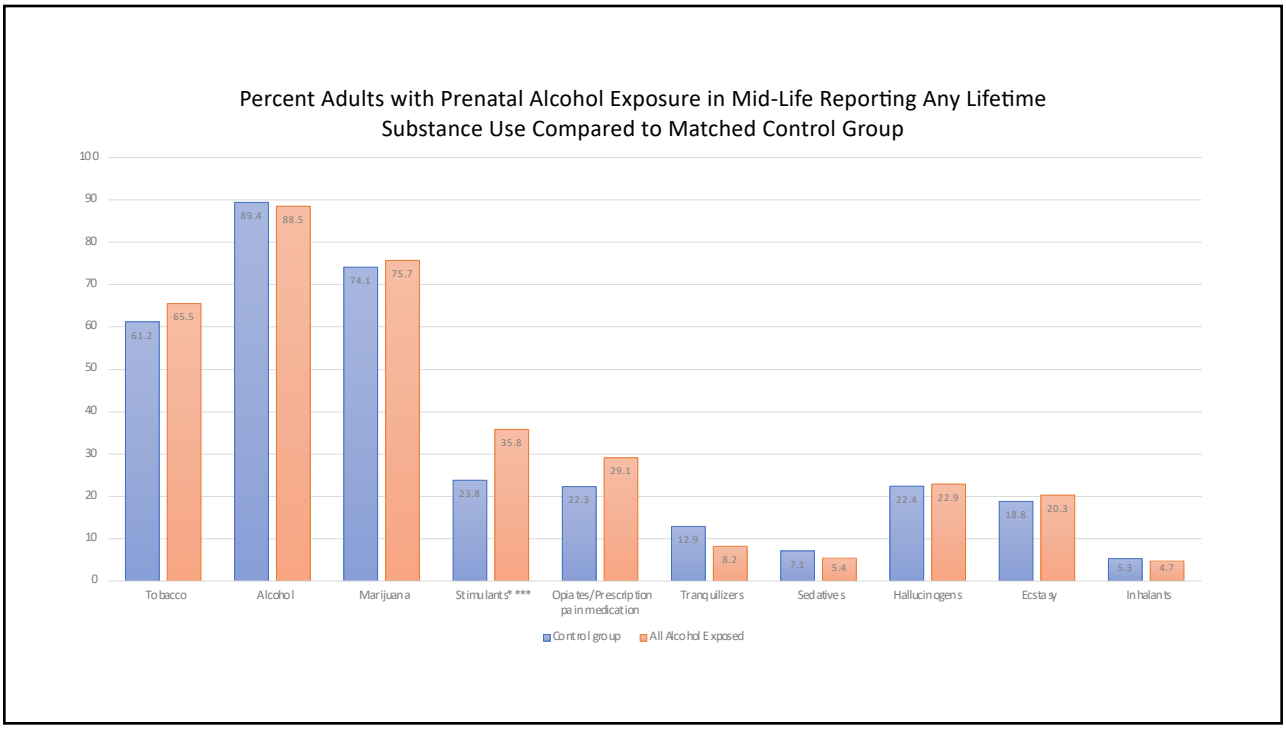
Characteristic	Control Group (n=85)	Alcohol Group (n=148)
Age at assessment	38.38 (5.1)	38.18 (6.16)
Sex at birth (% Female)	51%	55%
Race (% White/Black)	42.4/48.2%	35.8/49.3%
Marital Status (% Partnered)	41.7%	36%
Employment Status (FT/PT)*	75.3/16.5%	55.4/18.9%
NIH Tool Box Fluid IQ*	98.7 (23.2)	81.8 (17.5)
Hollingshead SES	40 (15.6)	28.65 (11.96)
Adverse Childhood Experiences (ACES)*	2.0 (2.1)	3.68 (2.91)
Positive Life T (LISRES)	65.8 (16.3)	63.1 (16.8)
Negative Life T (LISRES)	57.3 (14.0)	54.5 (13.1)

*Groups are significantly different at the $p < .001$ level

8

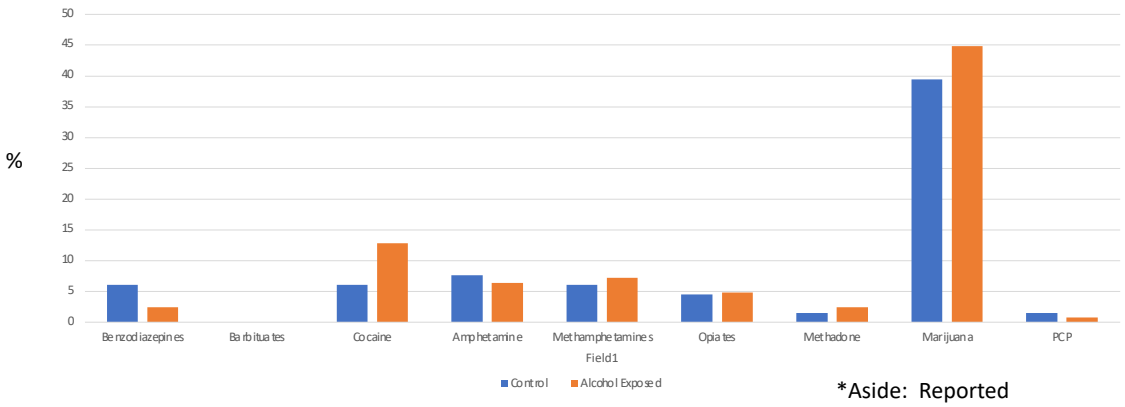

Percent Adults with Prenatal Alcohol Exposure in Mid-Life Reporting Current Substance Use Compared to Matched Control Group
90
80
70
60
50
40
30
20
10
0
22.4
39.9
82.4
64.2
37.6
48
11.8
18.2
3.5
2
1.2
0
7.1
0
2.4
0.7
2.4
0.7
1.2
1.4
Tobacco*
Alcohol**
Marijuana
Stimulants
Opiates/Rx pain medication
Tranquilizers
Sedatives
Hallucinogens
Ecstasy
Inhalants
Control group (n=85)
Alcohol Exposed (n=148)

9


Percent Adults with Prenatal Alcohol Exposure in Mid-Life Reporting Any Lifetime Substance Use Compared to Matched Control Group
100
90
80
70
60
50
40
30
20
10
0
61.2
65.5
89.4
88.5
74.1
75.7
23.8
35.8
22.3
29.1
12.9
8.2
7.1
5.4
22.4
22.9
18.8
20.3
5.3
4.7
Tobacco
Alcohol
Marijuana
Stimulants* ***
Opiates/Prescription pain medication
Tranquilizers
Sedatives
Hallucinogens
Ecstasy
Inhalants
Control group
All Alcohol Exposed

10

Results of Urine Screen for Control and Alcohol Exposed Groups (N=191): Percent Positive by Drug and Group



Trends for Cocaine (p=.113), and Benzodiazepines (p=.18)

*Aside: Reported Current Marijuana use almost identical to Urine screen results.

11

Further Analysis to determine factors influencing Substance Use

- Any Lifetime use
  - Tobacco
  - Alcohol
  - Marijuana
  - Opioids
  - Stimulants (Cocaine/Methamphetamine)
- Current Use
  - Tobacco
  - Alcohol
  - Marijuana
  - Opioids

- Generalized Linear Regression
- Factors and Covariates included
  - Prenatal Alcohol Exposure (2 and 3 groups)
  - Sex at birth (and, in this sample, currently)
  - SES (Includes job status and education)
  - ACEs
  - Current Stress/Resources-LISRES NLE

IQ not included as it is part of the Alcohol Effects Categorization. Race was not different. Age was not different.

12

Summary of Results

- Alcohol Use
  - AUDIT Total Score
    - Age, p=.05
    - ACEs, p=.05
  - GGT (Blood test)
    - ACEs, p<.006
    - Site, ATL>SEA, trend, p=.08
  - Ever Used Alcohol
    - nothing
  - Current Alcohol Use
    - Age, p=.02
    - SES, p=.002, Higher SES, more ALC

- Tobacco Use
  - Ever used Tobacco
    - Biological Sex, p<.004, M>F
    - Negative Life Events, trend, p=.06
  - Current Tobacco Use
    - Alcohol group, p=.005 (EtOH>Controls)
    - SES, p<.001, Higher, less use
    - Current Stress, p=.025
- Marijuana Use
  - Ever Used Marijuana
    - ACEs, p=.003
    - Biological Sex, p=.001 (M>F)
  - Current Marijuana Use
    - Biological Sex, p=.003, M>F
    - Age, p=.04, Young>Old
    - Site of data collection, p=.01, SEA>ATL

13

Summary of Results

- Stimulant Use
  (Cocaine, crack, methamphetamines)

- Ever Used Stimulants
  - ACEs, p=.008
  - Biological sex, p=.048, F>M
- Current Stimulant Use
  - No factors accounted for significant variance.

- Opioid Use
  - Ever used Opioids
    - Site of data collection, SEA>ALT

  - Current Opioid Use
    - None reported or detected in urine screens

Ns for these drugs were small. PAE not significant when covariates controlled.

14

PLANS FOR FY 2024

- Finalize transfer of PI and subcontract to Calgary
- Continue ongoing data collection
- Transmit samples when interim goals reached
- Dissemination
  - Presentation at Seattle FASD Meeting
  - RSA
  - Complete papers

15

15

A multisite study of prenatal alcohol exposure: Effects of inflammation and endocrine dysfunction in adulthood

Drs. Tamara Bodnar, Charlis Raineki, Joanne Weinberg

Canadian Update

December 15, 2023

1

CIFASD U01: Canadian Update

<u>Project update:</u>

- MPI change
- Subcontract moving to UCalgary (*in progress*)

UBC

UNIVERSITY OF CALGARY

2

CIFASD U01: Canadian Update

<u>Ethics & Protocols:</u>

- Re-submission to UCalgary ethics (**IRB**): Final approval pending (estimated Jan 2024)
- **Dysmorphology**:
  - Training w/ Miguel (first session complete)
  - Dysmorphology training session: n=14 exams
- Established contract with Heritage Medical Research Clinic and Alberta Health Services

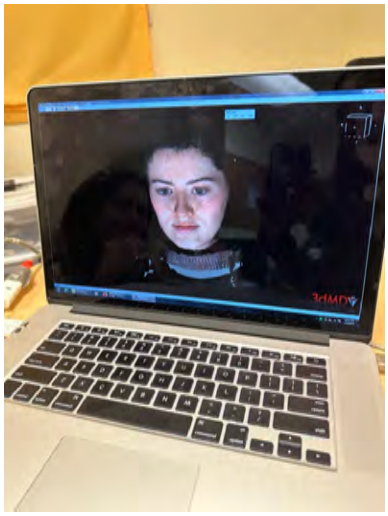
UNIVERSITY OF CALGARY

3

CIFASD U01: Canadian Update

<u>Protocols:</u>

- 3D image collection:
  - Collaboration with Dr. Hallgrimson at UCalgary (access to 3dMDface system at Alberta Children's Hospital)
  - Training session complete and pilot images collected



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CIFASD U01: Canadian Update

DCR update :

- Data dictionary complete
- Practice data submitted; approved
- Data upload: exemption submitted (IRB approval pending)
- GUID training: Complete, access pending

5

CIFASD U01: Canadian Update

Study Team:

- Research assistant (Kennedy Howatt)
- Research assistant 2 (in progress)
- Adult with living experience
- Forming a study advisory circle (including Indigenous perspective)
- UCalgary and U of A mentors: Drs. Lebel, Pei, McMorris, Badry

6

CIFASD U01: Canadian Update

Pre-Recruitment: Goal n=42 enrolled & tested by end of year 2

- Virtual study subjects: consented to be re-contacted (n=28)
- **CanFASD 2023 Conference** (n=20 participants expressed interest)
- Calgary Fetal Alcohol Network, Glenrose FASD clinic, Foothills FASD Assessment & Diagnostic Clinic & 24 additional community organizations and clinics
- **FASD Conference in Seattle** (2024): Will recruit subjects
- Recruitment collaborations with Drs. Lebel, Pei, & McMorris
- Older control group: Two neurologists at UCalgary to support
- Indigenous partners at UCalgary and community

CFAN Calgary Fetal Alcohol Network

7

CIFASD5 Canadian Update

Interactions with other projects:

- Mahnke: n=12 subjects with collection of samples prioritized; protocols tested
- Suttie: 3D images
- DelCampo: Dysmorphology & MorpheusQ training
- Petrenko/Tapparello: Will share study info for recruitment
- Miranda: Protocol in place for sample collection (telomere length)

8

CIFASD5 Canadian Update

Publications and presentations:

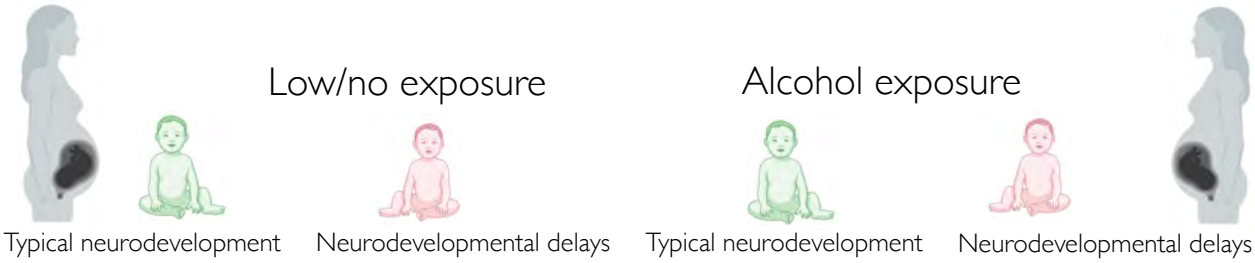
- Bodnar, T.S., Chao, A., Homan, P.J., Ellis, L., Raineki, C., & Weinberg, J. (2023) Impact of the COVID-19 pandemic on adults with Fetal Alcohol Spectrum Disorder: linking immune function to mental health status. *Front. Neurosci.* 17, 1-10.
- CanFASD Presentation: Evaluating the Impact of Prenatal Alcohol Exposure on Adult Health: Evidence for Risk and Resilience
- Upcoming FASD Seattle Presentation: Exploring health outcomes in adults with FASD: Evidence from the Canadian research study site
- Upcoming RSoA Presentation: Prenatal Alcohol Exposure Results in Long-Lasting Alterations in Immune Function: Evidence from a Multi-Site Study

9

CIFASD5 Canadian Update

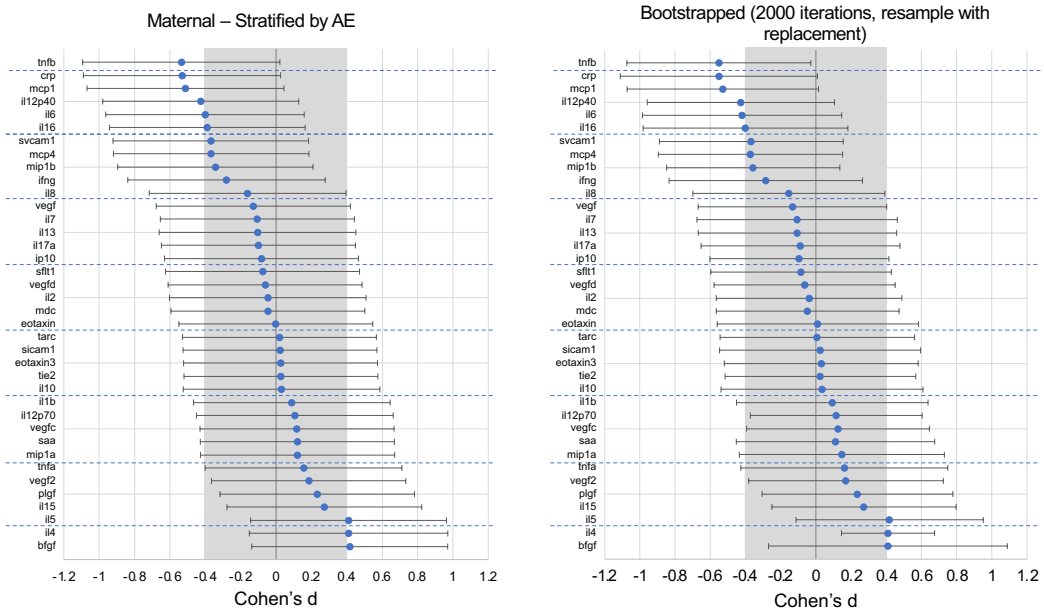
Maternal-Child Cytokine Data Update: Collab. w/ Amanda Mahnke

- Collab. with Tina Chambers: samples from Ukraine cohort
- Analysis collab. with Amanda Mahnke



10

Although smaller N, initial effect size estimates are stable



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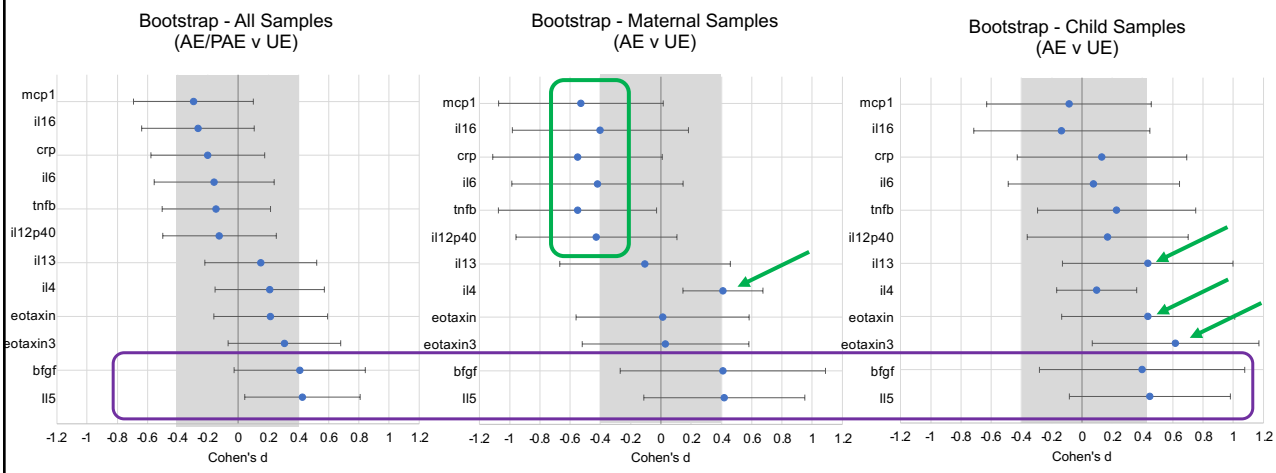
Maternal and child samples have differing cytokine signatures

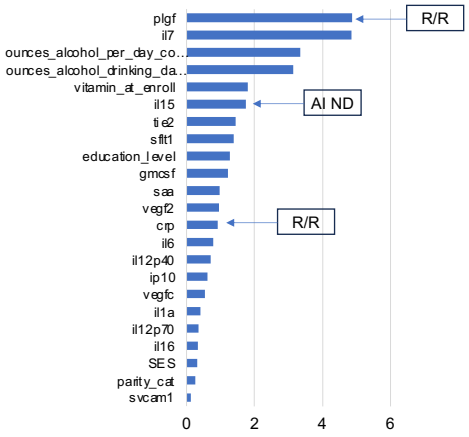


12

RFA improves when discerning between PAE- and non-PAE-associated neurodevelopmental delay

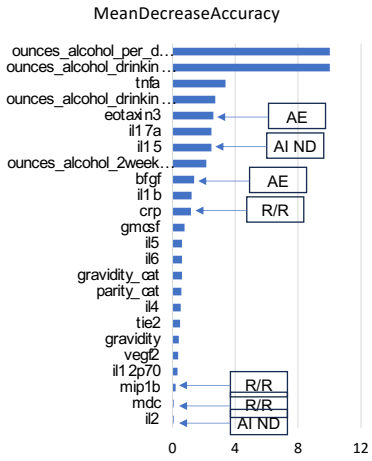
Child (stratified by neurodevelopmental status)

OOB: 47.27%



Child with Neurodevelopmental Delay (stratified by exposure)

11.11%



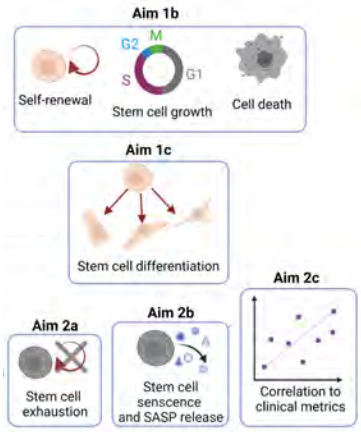
Bodnar et al., 2020 Networks	
R/R	Alcohol Risk/Resiliency
AI ND	Alcohol-Independent Neurodevelopmental Delay
AE	Alcohol Exposure

13

Lifelong impact of PAE on stem cell dynamics and cellular aging

UH2AA030186
PI: Amanda Mahnke

- **Aim 1 - Does PAE diminish stem cell function across the lifespan?**
- Progress:
  - Two contrast samples – commercially available adult PBMCs
  - Receiving child samples from Chambers/UCSD
- **Aim 2 - Does PAE induce or exacerbate stem cell aging?**
- Progress:
  - Assays created for qPCR metrics of aging, including potential normalization transcripts
  - Piloting flow cytometry assays for markers of aging



1

Lifelong impact of PAE on stem cell dynamics and cellular aging

UH2AA030186
PI: Amanda Mahnke

Mahnke Lab Updates

- IRB (exempt/not human subjects – Oct 2022)

Data Coordination Resource

- Data dictionary created and approved
- Data dictionary finalized and portal created
- Ready to upload data as it is created

Contrast Adult hiPSCs

- ~40 yo
- Male – induced and undergoing expansion
- Female – induction ongoing

2

Samples Update

Chambers - child samples (UCSD)

- Collection on going
- 3 samples, 6-12 years of age, 1 male and 2 females, 2 PFAS and 1 PAE
- Can examine PAE v FASD but also in discussions with Elizabeth/Bill about potential unexposed contrast samples

Coles/Weinberg/Bodnar/Raineki – adult samples (Canada)

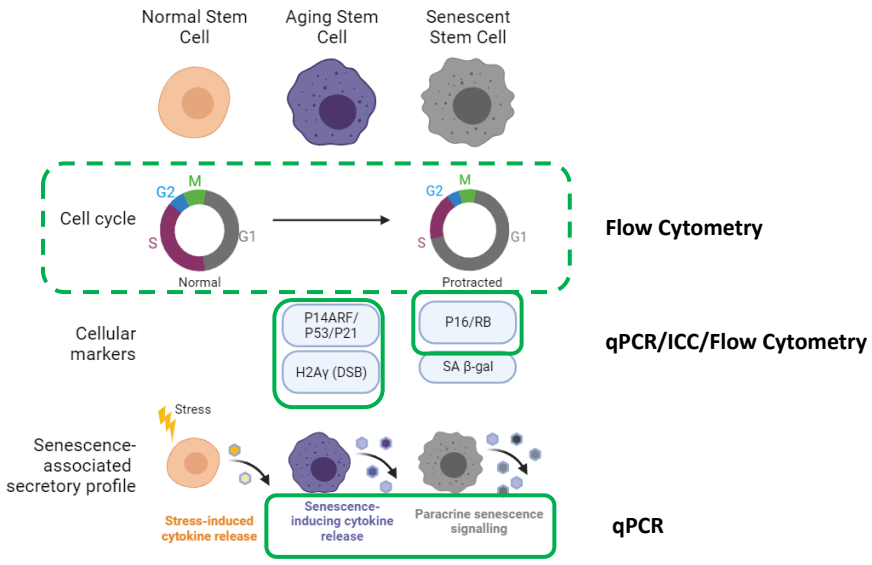
- Finalizing ethics review
- PBMC samples for UH2 study will be prioritized when collection begins

DiClemente/Stotts – neonate samples (UH)

- Planning to collect samples from pilot cohort

3

Major Accomplishments: Designing/Validating SASP and Senescence Assays



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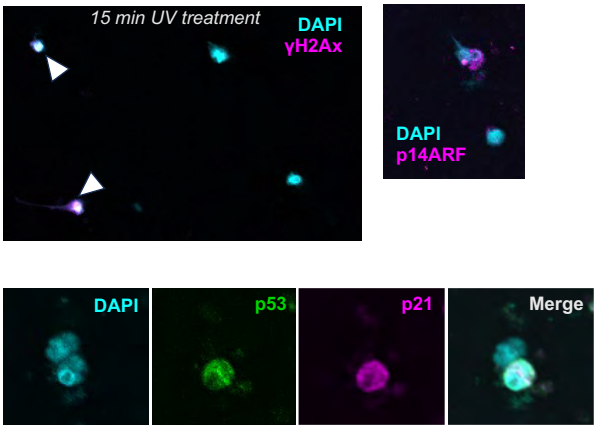
Designing/Validating SASP and Senescence Assays

qPCR panel for SASP and Senescence-Complete

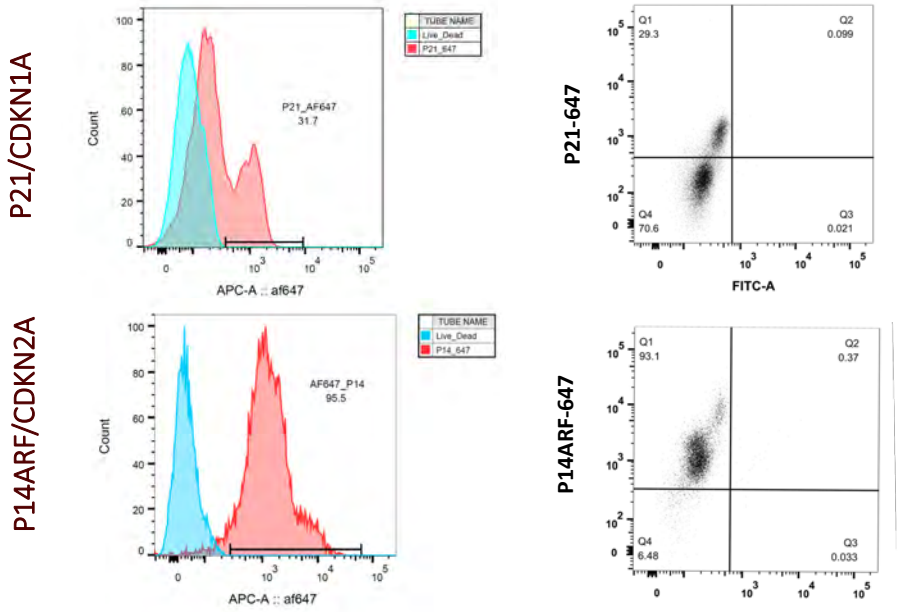
Gene Target	Role	Sequenced	
IL-6	SASP	Yes	Validated
VEGFA	SASP	Yes	Validated
CXCL8	SASP	Yes	Validated
IL-1A	SASP	Yes	Validated
IL-7	SASP	Yes	Validated
CXCL8 (IL-8)	SASP	Yes	Validated
CSF2(GM-CSF)	SASP	Yes	Validated
VEGFC	SASP	Yes	Validated
GLB1	Senescence	Yes	Validated
p21/CDKN1A	Senescence	Yes	Validated
p16INK4A/CDKN2A	Senescence	Yes	Validated
p14ARF/CDKN2A	Senescence	Yes	Validated
CDKN2B	Senescence	Yes	Validated
LMNB1	Senescence	Yes	Validated
TP53	Senescence	Yes	Validated
NOTCH1	Senescence	Yes	Validated
B2M	Housekeeping	Yes	Validated
HPRT1	Housekeeping	Yes	Validated
GAPDH	Housekeeping	Yes	Validated
ACTB	Housekeeping	Yes	Validated
ATP5B	Housekeeping	Yes	Validated
PGK1	Housekeeping	Yes	Validated

- Immunomarkers for SASP and Senescence



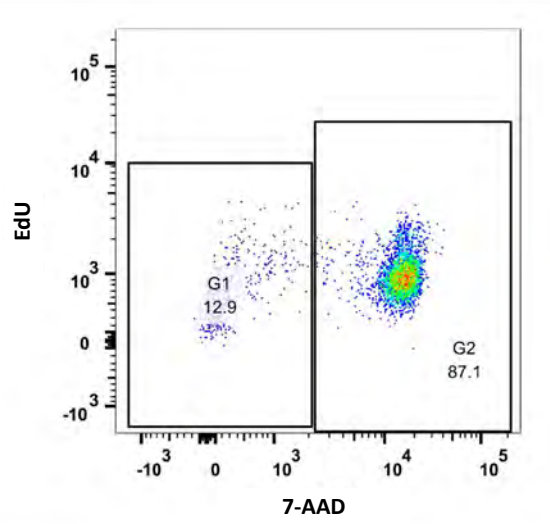
5

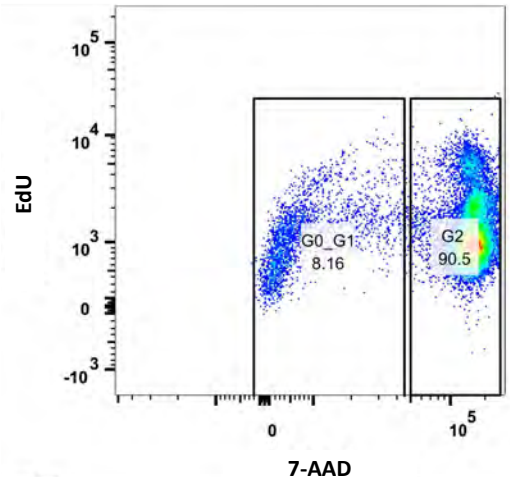
Flow Cytometry – Aging/Early Senescence



6

Flow Cytometry – Cell Cycle (*in progress*)





7

Additional CIFASD collaborations

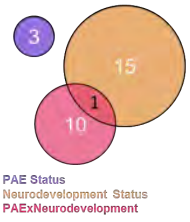
Weeks

- 12mo kidney marrow to assess markers of aging
- Quick Pass of 5.5/6mo cardiac RNAseq

Upregulated	*Downregulated*
scpep1	atf3
lyve1a	hadh
coro1a	irs2b
c6	grasp
vim	myh6
phlda3	acss1
osr1	nnt
rpl13a	mccc2
lum	ube2ql1
arpc1b	tob1b
	midn
	nr4a1
	zfand5b
	acsf2
	cdnf
	tcp11l2
	lpin1
	slc38a2

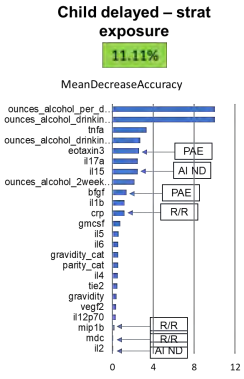
Chambers/Miranda

- Assessment of child miRNAs and maternal/child dyads
- RSA 2024 symposium



Weinberg/Bodnar/Raineki

- Assessment of maternal/child dyad cytokines



Suttie

- Machine Learning Narrative Review for ACER (revised and resubmitted)

8

Goals for April

- hiPSC creation and assessments started for child and adult samples
- In the middle of collection of neonate samples from DiClemente/Stotts pilot cohort
- NCE to continue the work/analysis

9

Future Directions

- Analysis of transcripts in hematopoietic niche of 5mo rat and 15mo zebrafish models of PAE
  - Potential supporting evidence for human stem cell data
  - May identify additional assessments
- Therapeutics for PAE-induced stem cell aging grant
  - Potential therapeutic identification with supporting preclinical models
- Assessment panel for peripheral stem cells (CD34+ PBMCs) for PAE-induced precocious stem cell aging

10

Development of biomarkers in deciduous teeth of children with FASD that predict neurobehavioral performance

1 UH2 AA029062

PIs: Annika Montag & Manish Arora

CIFASD Midyear Meeting December 17th, 2023

UC San Diego School of Medicine

Rady Children's Hospital San Diego

Icahn School of Medicine at Mount Sinai

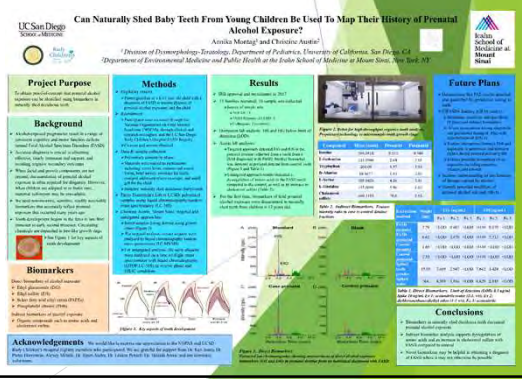
Specific Aims

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 deciduous 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.

Aim 4. (Added Aim from R21) Explore potential biomarkers of co-exposures including cannabis, tobacco, and opioids.



Progress & Future Plans

Progress

- Recruitment has been slow: 22 participants (17E, 5U) of 25E/25U goal recruited
- Methods have been more difficult to establish than anticipated necessitating additional recruitment under our pilot study IRB approvals and more time spent on methods
- CIFASD samples (34 teeth, 22E/12U) and non-CIFASD samples (43 teeth, 21E/20U) sent to Mt Sinai
- PAE biomarkers: EtG, EtS, FAEE (EM, EP, ES, EO) and PEth (1 and 2) assessed
  - Table 1. Biomarkers shown by trimester including second run of EtG and EtS
  - Figure 1. EtG and EtS data in figure format to show relative magnitudes and changes over time
  - TAble 2: EtG and EtS first and second run for comparison
  - Figure 2. Metals analysis on samples from 15 exposed and 5 unexposed participants
- MPI change initiated: Welcome Dr. Manish Arora

Future Plans

- Decision: whether methods are established or require more work by end of year
- Assess and analyze
  - Previously unassessed samples for EtG, EtS, FAEEs, and PEth
  - all samples for indirect biomarkers: amino acids and cholesterol sulfate
  - all samples for co-exposures: cannabis, tobacco, opioids
- Obtain neurobehavioral data from Mattson and Wozniak
- Analysis of associations of exposures and NB outcomes
- Methods and associations manuscripts
- R01 submission

Other to date

- All IRB materials and approvals for CIFASD and pilot study recruitment are valid through May 2024
- One manuscript: POC; Two conference presentations/ posters; One mention in popular literature "The Truth's in the Tooth" Analytical Scientist 2022

Table 1. Biomarkers of Perinatal Alcohol Exposure

6 exposed (orange shading) and 4 unexposed (green shading) donors (pg/mg tooth)

2T								3T								Post							
EtG	EtS	EM	EP	ES	EO	PEth 1	PEth 2	EtG	EtS	EM	EP	ES	EO	PEth 1	PEth 2	EtG	EtS	EM	EP	ES	EO	PEth 1	PEth 2
								0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
38.7	28.2	0.0	0.0	0.0	0.0	0.0	0.0	42.7	11.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
								0.8	2.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
84.5	16.9	0.0	0.0	549.1	0.3	0.0	0.0	0.0	0.0	0.1	41.1	1037.6	16.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4.6	0.0	0.0	129.2	3590.3	0.0	0.0	0.0	5.4	0.0	0.0	0.0	220.2	0.0	0.0	0.0	165.9	10.0	7.5	93.4	140.4	19.7	0.0	0.0
9.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	46.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16.0	165.1	0.0	0.0	0.0	0.0	0.0	0.0	128.6	54.2	0.0	0.0	0.0	0.0	0.0	0.0	51.6	37.5	0.0	0.0	0.0	0.0	0.0	0.0
30.8	24.0	0.0	79.5	1100.5	0.0	0.0	0.0	0.0	0.0	0.0	73.9	977.7	4.1	0.0	0.0	0.0	0.0	0.0	0.0	91.8	1.0	0.0	0.0
								108.5	7.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
67.1	25.7	0.0	365.8	2157.7	0.0	0.0	0.0	44.6	12.4	13.6	19.5	502.0	2.1	0.0	0.0	19.1	4.0	1.4	33.4	31.8	1.3	0.0	0.0

EtG			EtS			Ethyl myristate (EM)			Ethyl palmitate (EP)			Ethyl stearate (ES)			Ethyl oleate (EO)			PEth 16:0/18:1 (PEth 1)			PEth 16:0/18:2 (PEth 2)		
2T	3T	Post	2T	3T	Post	2T	3T	Post	2T	3T	Post	2T	3T	Post	2T	3T	Post	2T	3T	Post	2T	3T	Post
	0.0	0.0		0.0	0.0		0.0	0.0		0.0	0.0		0.0	0.0		0.0	0.0		0.0	0.0		0.0	0.0
38.7	42.7	0.0	28.2	11.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.8	0.0		2.0	0.0		0.0	0.0		0.0	0.0		0.0	0.0		0.0	0.0		0.0	0.0		0.0	0.0
84.5	0.0	0.0	16.9	0.0	0.0	0.0	0.1	0.0	0.0	41.1	0.0	549.1	1037.6	0.0	0.3	16.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4.6	5.4	165.9	0.0	0.0	10.0	0.0	0.0	7.5	129.2	0.0	93.4	3590.3	220.2	140.4	0.0	0.0	19.7	0.0	0.0	0.0	0.0	0.0	0.0
9.2	46.9	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16.0	128.6	51.6	165.1	54.2	37.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
30.8	0.0	0.0	24.0	0.0	0.0	0.0	0.0	0.0	79.5	73.9	0.0	1100.5	977.7	91.8	0.0	4.1	1.0	0.0	0.0	0.0	0.0	0.0	0.0
	108.5	0.0		7.5	0.0		0.0	0.0		0.0	0.0		0.0	0.0		0.0	0.0		0.0	0.0		0.0	0.0
67.1	44.6	19.1	25.7	12.4	4.0	0.0	13.6	1.4	365.8	19.5	33.4	2157.7	502.0	31.8	0.0	2.1	1.3	0.0	0.0	0.0	0.0	0.0	0.0

Figure 1. Biomarkers of Perinatal Alcohol Exposure

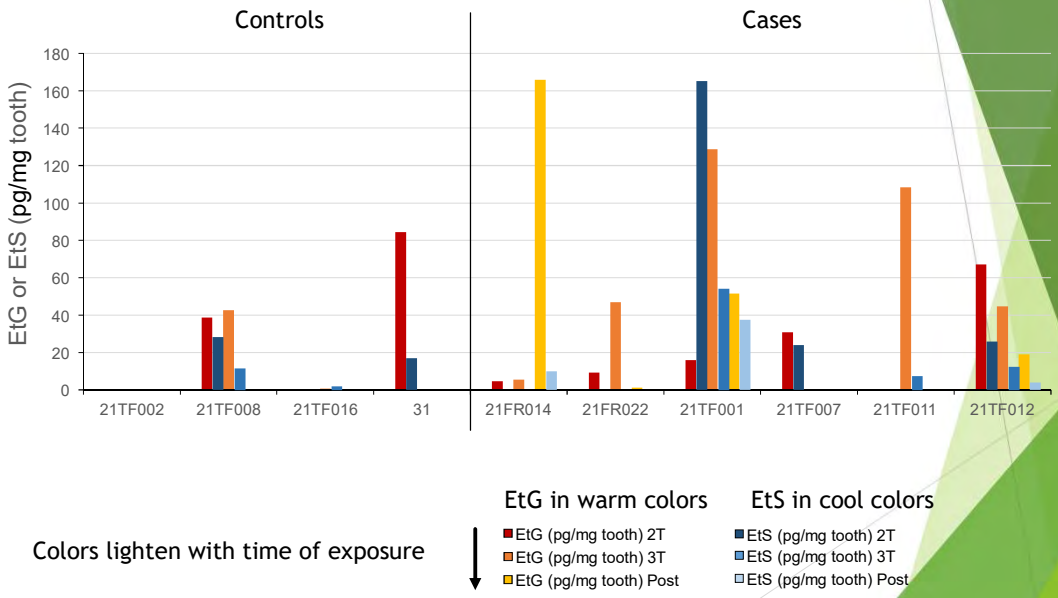


Table 2: EtG and EtS Biomarkers Comparing First and Second Runs

First run

	2nd Trimester		3rd Trimester		Postnatal	
Donor	EtG (pg/mg tooth)	EtS (pg/mg tooth)	EtG (pg/mg tooth)	EtS (pg/mg tooth)	EtG (pg/mg tooth)	EtS (pg/mg tooth)
Control 1	-	-	0.00	0.00	0.00	0.00
Control 2	0.00	28.16	0.00	11.62	0.00	0.00
Control 3	-	-	0.00	85.39	0.00	0.00
Control 4	164.29	0.00	0.00	0.00	0.00	0.00
Case 1	66.89	0.00	84.52	0.00	159.63	12.18
Case 2	73.20	0.00	31.12	0.00	8.45	0.00
Case 3	0.00	230.49	0.00	55.88	0.00	43.73
Case 4	0.00	21.09	0.00	0.00	0.00	0.00
Case 5	-	-	0.00	16.96	0.00	0.00
Case 6	0.00	87.01	0.00	12.35	0.00	3.91

Second run

	2nd Trimester		3rd Trimester		Postnatal	
Donor ID	EtG (pg/mg tooth)	EtS (pg/mg tooth)	EtG (pg/mg tooth)	EtS (pg/mg tooth)	EtG (pg/mg tooth)	EtS (pg/mg tooth)
Control 1			0.00	0.00	0.00	0.00
Control 2	38.73	28.16	42.67	11.62	0.00	0.00
Control 3			0.79	2.05	0.00	0.00
Control 4	84.51	16.87	0.00	0.00	0.00	0.00
Case 1	4.63	0.00	5.45	0.00	165.91	9.99
Case 2	9.23	0.00	46.91	0.00	1.09	0.00
Case 3	16.01	165.07	128.65	54.19	51.56	37.51
Case 4	30.81	24.04	0.00	0.00	0.00	0.00
Case 5			108.49	7.49	0.00	0.00
Case 6	67.09	25.72	44.59	12.35	19.11	4.02

Figure 2: Metals Analysis

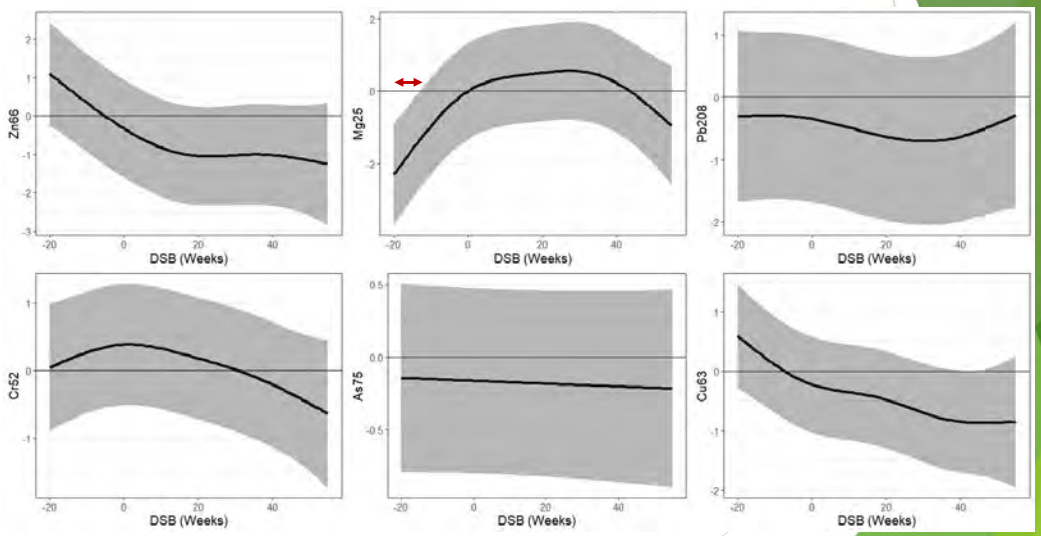
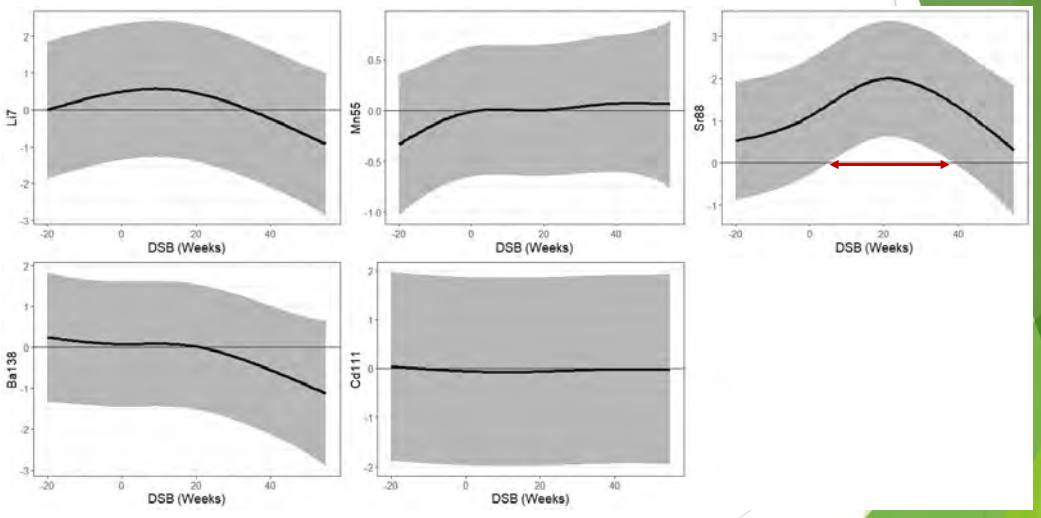
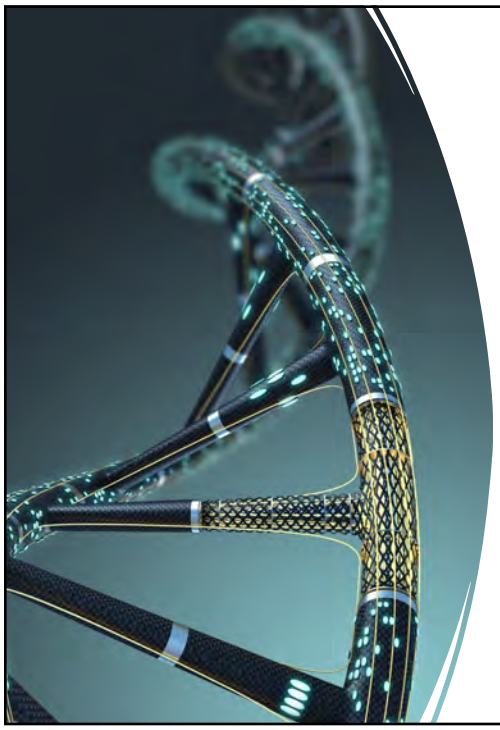


Figure 2 continued: Metals Analysis





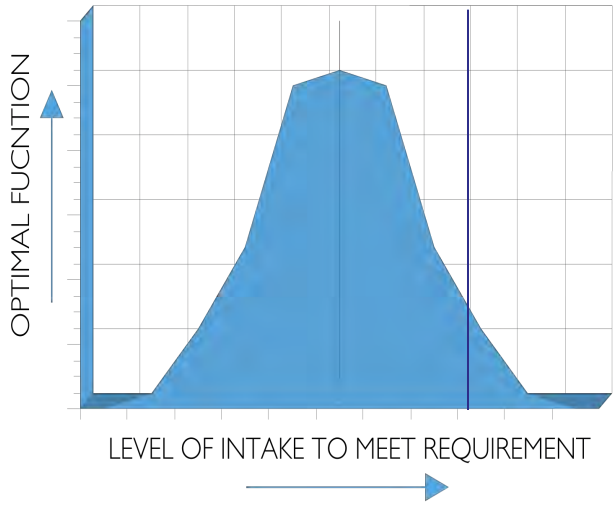
Polymorphisms in choline metabolism affect the cognitive impact of Prenatal Alcohol Exposure

Susan M. Smith, Ph.D.
UNC Nutrition Research Institute
Kannapolis NC
Susan_Smith@unc.edu

UNC NUTRITION RESEARCH INSTITUTE

1

We assume people are average



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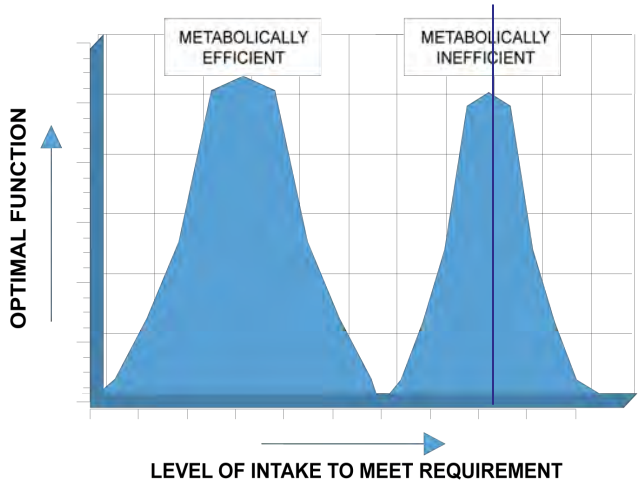
EATUNIQUELY

2

But there are people that differ in nutrient requirements & responses



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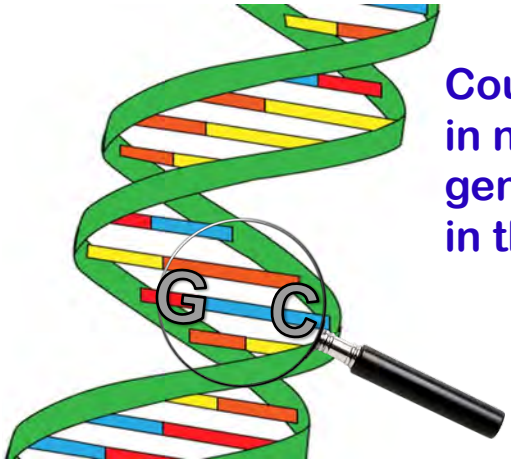

Could allelic differences in nutrient-related genes affect outcomes in those with FASD?

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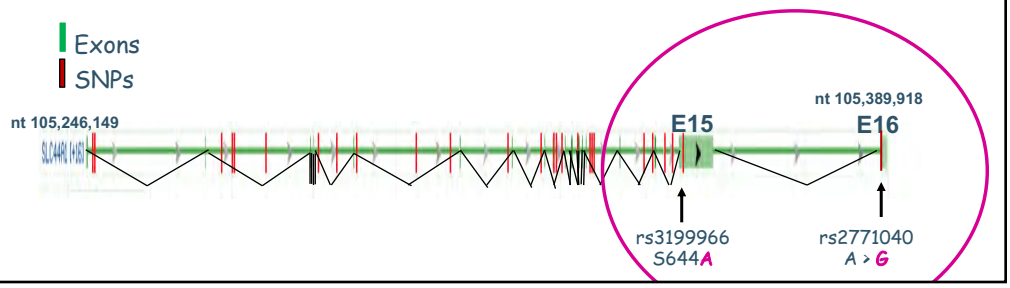
4

SLC44A1 – primary choline transporter

- Functional loss: cognitive decline, ataxia, white matter deficits, cerebral & cerebellar atrophy
- rs3199966(T>G) – S644A, Exon 15, C-terminal
  - ***GG*** associated w/increased vulnerability to choline deficiency[1]
  - High choline intake: TG/GG directs more choline into methylation pathway (betaine and methionine) [2]
- rs2771040 (A>G) – 3'UTR, plasma membrane isoform
  - ***GG*** associated w/increased vulnerability to choline deficiency[1]

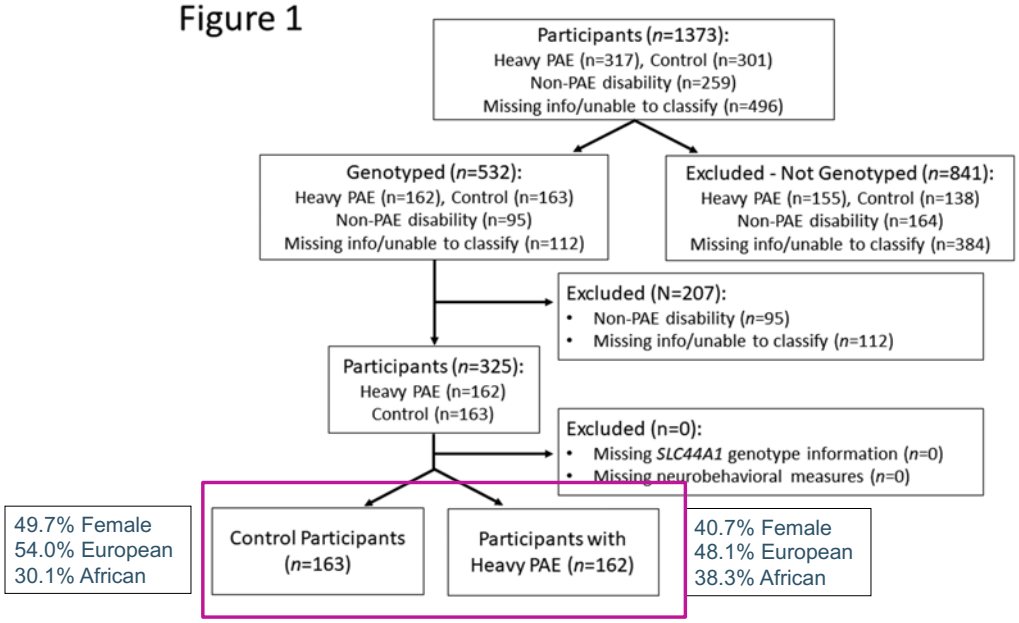


[1] da Costa et al. 2006, 2014
[2] Ganz et al. 2016, 2017

5

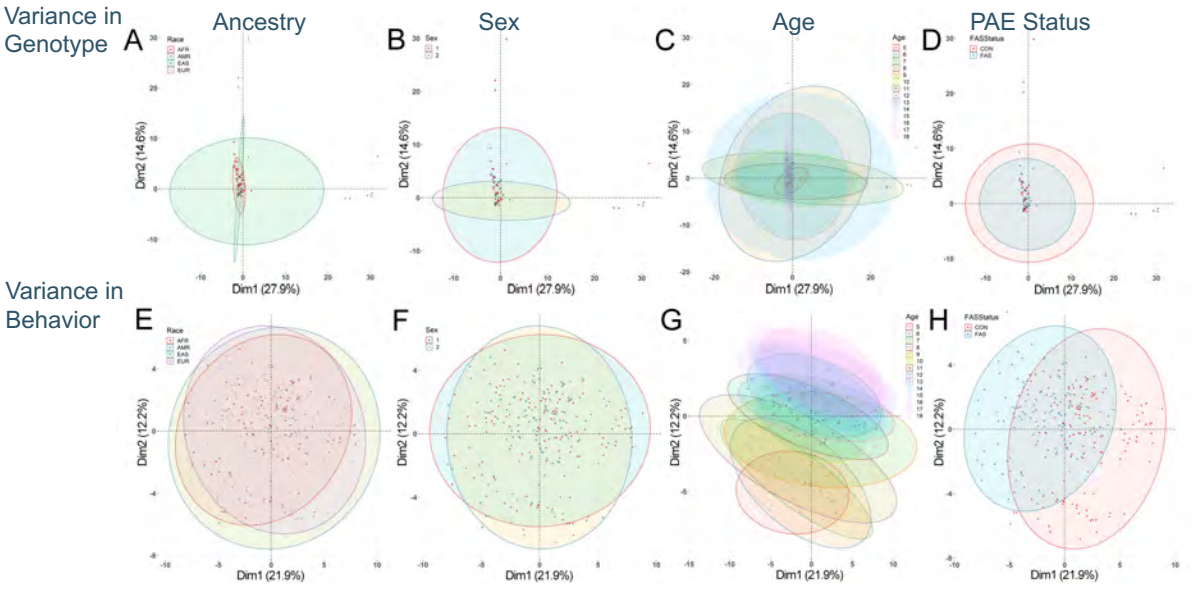
Aim 2 – CIFASD2/3 Participants

Figure 1



6

PAE Status and Age Explain the Behavioral Variance



7

8 SNPs in *SLC44A1* are Associated with at Least One Behavioral Outcome

ID	Location	Type	Ref/Alt	MAF, this cohort	MAF, European	MAF, African Amer.	Function
rs75106836	Intron 1	SNV	T > C	2.9%	0.04%	5.5%	unknown
rs105185127	Intron 1	SNV	C > T	3.7%	0.8%	11.0%	unknown
rs143438338	Intron 1	SNV	A > G	3.0%	0.04%	5.5%	unknown
rs59370172	Intron 1	SNV	C > T	3.0%	0.04%	5.6%	unknown
rs12347364	Intron 1	SNV	T > A	5.6%	5.0%	0.7%	unknown
rs10991629	Intron 3	SNV	C > T	18.7%	11.8%	36.3%	unknown
rs3199966	**Exon 15**	**SNV**	**T > G**	**19.1%**	**9.0%**	**41.5%**	**increases choline need**
rs2771040	**Exon 16 (3' UTR)**	**SNV**	**A > G**	**21.3%**	**12.0%**	**43.8%**	**increases choline need**

Smith et al. *Am J Clin Nutr* (in press)

8

Associations w/Executive Function, Memory, Learning, & Reasoning

Cognitive Measure	SNP	ADDxPAE Q-Value
Perseverations (Free and Cued Recall Total), Z-score (CVLT)	rs150185127	0.0200
Serial Cluster Ratio, Z Score (CVLT)	rs12347364	0.0116
Matrices (DAS-II)	rs59370172	0.0499
Conners Executive Functioning	rs2771040	0.0116
General Cognitive Abilities (DAS-II)	**rs3199966**	**0.0116**
	rs75106836	0.0147
Nonverbal Reasoning Cluster (DAS-II)	**rs3199966**	**0.0114**
	rs2771040	**0.0117**
	rs10991629	0.0118
	rs12347364	0.0146
Recall of Designs (DAS-II)	**rs3199966**	**0.0204**
	rs2771040	**0.0213**
	rs10991629	0.0145
Sequential & Quantitative Reasoning (DAS-II)	**rs3199966**	**0.0113**
	rs2771040	**0.0113**
Internalizing behavior, V-score (VABS-2)	rs3199966	0.0116

Smith et al. *Am J Clin Nutr* (in press)

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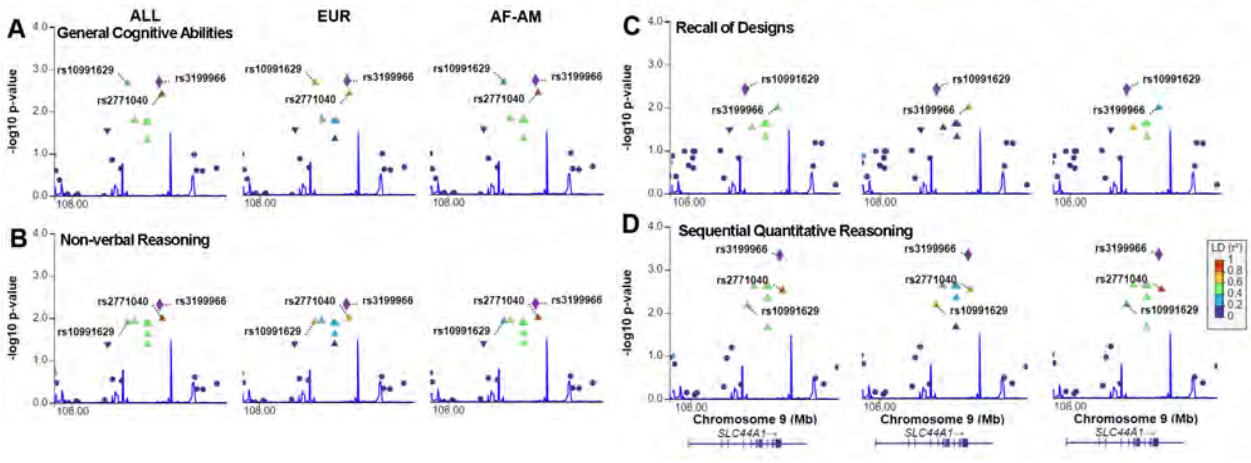
SNPs in LD w/rs3199966 are Associated with These Impairments



Smith et al. *Am J Clin Nutr* (in press)

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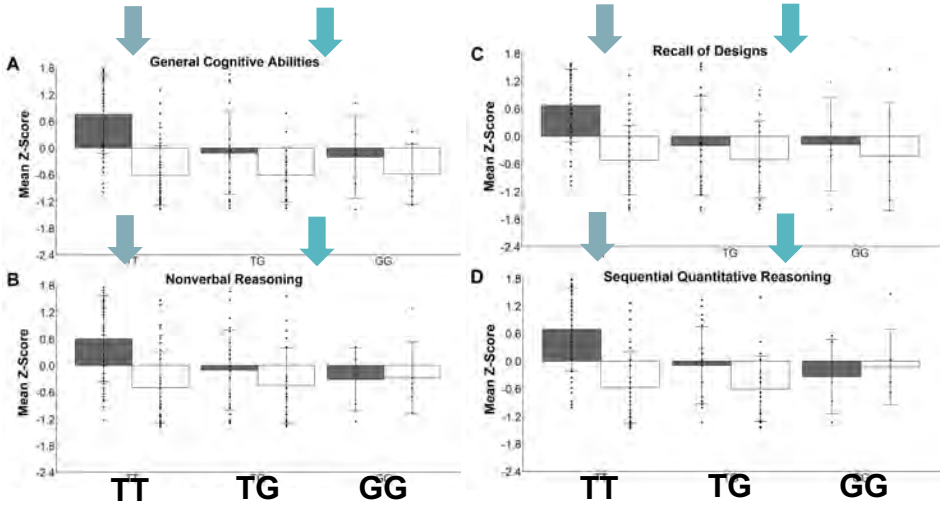
When extra choline is absent, GT/GG carriers have worsened cognitive performance.

Control
PAE



Smith et al. *Am J Clin Nutr* (in review)

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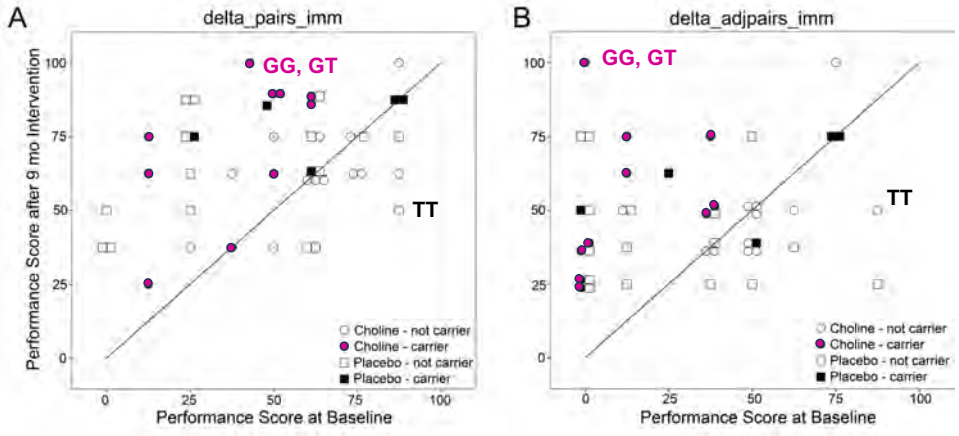
Wozniak Choline Intervention: GT/GG carriers have greater pre/post cognitive improvement in 9-mo choline intervention

EI memory task – pairs in order

EI memory task – pairs any order



Smith et al. *Am J Clin Nutr* 2021

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Other Aims

- Aim 1 – Rinse & Repeat in OmniNet Cohort
  - Sequences (finally!) released in May 2023 from Kids First
  - Obtained approval from dbGaP
  - In the midst of data cleansing prior to association analysis
- Aim 3 - CRISPR SLC44A1 to study function in SH-SY5Y neuroblastoma
  - rs3199966 (C>G)
  - rs2771040 (A>G)

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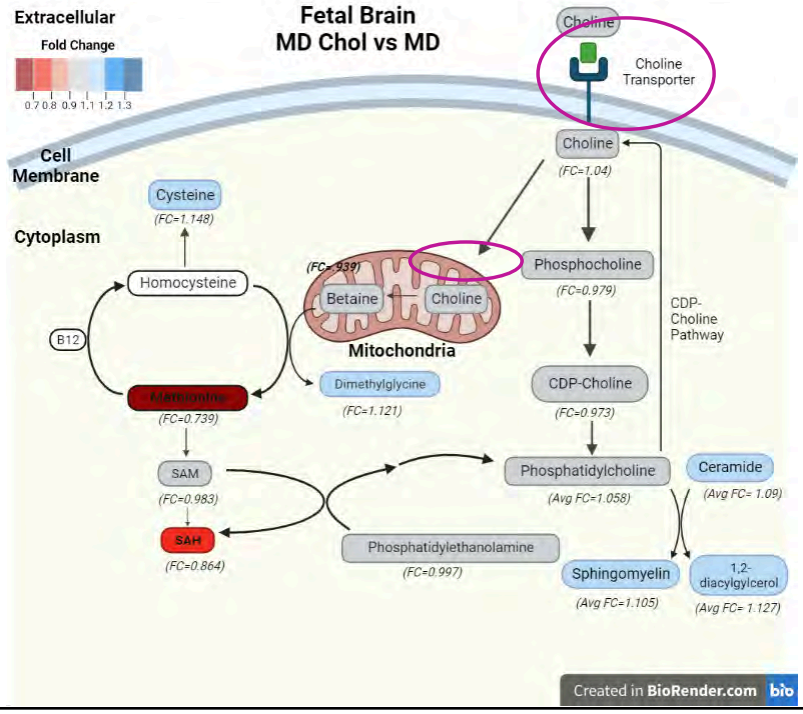

It's not just *SLC44A1*.

Many genes impact choline & 1-Carbon metabolism.

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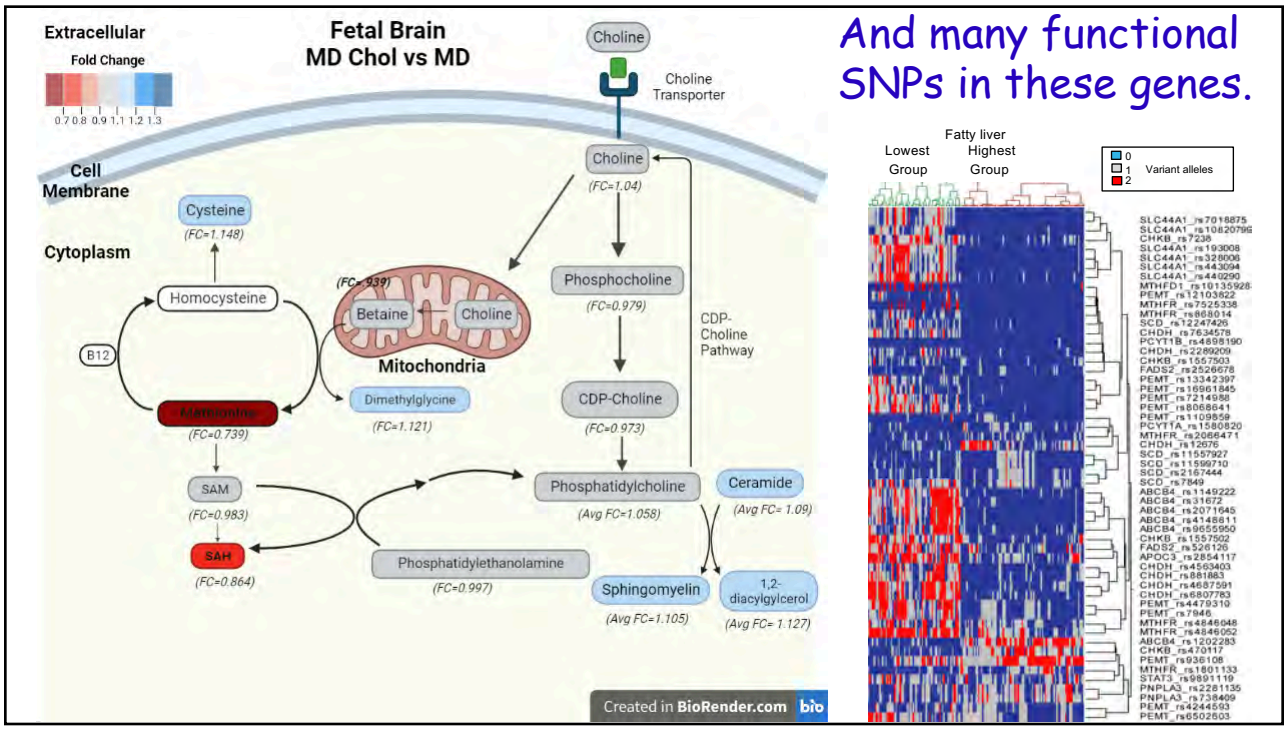

And many functional SNPs in these genes.

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Wozniak Choline Intervention: 1C SNP Associations in All Participants

Gene	SNP	Q-Value	Outcome	Effect Allele (Major > Minor Allele)
BHMT	rs558133	0.00076	iib_tscore13	Increased with G (T > G)
	rs558133	0.00041	iib_tscore20	Increased with G (T > G)
	rs567754	0.01236	weight_z_physical.1	Increased with C (C > T)
	rs567754	0.00341	weight_pile_physical.1	Increased with C (C > T)
FMO3	rs2064074	0.04245	weight_pile_physical.3	Increased with A (A > G)
MTHFR	rs4846048	0.00809	t_score3.1	Increased with A (A > G)
	rs17421511	0.04828	stanfbi_qr	Increased with G (G > T)
	rs6667720	0.00809	t_score3.1	Increased with T (T > C)
SHMT1	rs1979277	0.00565	t_score8.11	Increased with G (G > A)

Smith et al. *Am J Clin Nutr* (2021)

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Choline-related Genes Associated w/General Cognitive Abilities in CIFASD2/3

Gene	Gene Name/Function	Alt Freq	P-adj	β ± SE	Effect Size	Effect on Choline
SLC44A1	Choline Transporter CTL1	21.7%	0.0056	-0.72 ± 0.18	3.43	↑ Need
ALDH1L1	Cytosolic 10-Formyl THF Dehydrogenase	5.3%	3.43E-4	+0.79 ± 0.19	3.76	↓ Need
DMGDH	Dimethylglycine Dehydrogenase	19.1%	4.21E-4	+1.12 ± 0.33	3.63	↓ Need
MTHFD1L	10-formyl-THF Synthetase	8.6%	3.24E-4	-0.81 ± 0.22	3.89	↑ Need

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Gene x Exposure associations (ADD model) for ALDH1L1 variant rs3796191(T>C) (L254P).

Behavioral Measure	Beta ± SE	Effect Size	p-adj	Minor Allele Effect
General Cognitive Fcn [a]	+0.89 ± 0.21	4.80	0.0003	Better performance
Seq Quant Reasoning [a]	+0.78 ± 0.19	4.19	0.0012	Better performance
Global Executive Fcn [b]	+0.94 ± 0.27	3.43	0.0184	Better performance
Nonverbal Retrieval [c]	+1.25 ± 0.33	3.79	0.0065	Better performance
Free-Cued Recall [d]	1.06 ± 0.26	3.99	0.0025	Better performance
ADD inattentive type [e]	-1.09 ± 0.26	4.20	0.0011	Fewer problems
Monitor [b]	-0.98 ± 0.27	3.59	0.0104	Fewer problems
Emotional Regulation [b]	-1.06 ± 0.30	3.54	0.0124	Fewer problems

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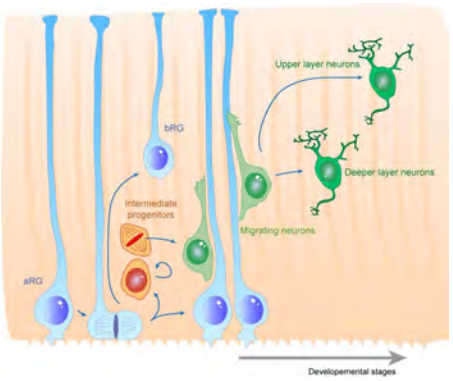
What is *ALDH1L1*?

- 10-formyl-THF + $NADP^+$ → THF + CO_2 + NADPH
- Controls cellular Methyl pools
- Induced by PAE, normalized by choline
- Expressed in radial glia
- Then restricted to astrocytes
- KOs have behavioral deficits



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Deliverables

- Publ

- R01 AA031262 – "Choline Polymorphisms in FASD"
  Resubmitted Nov 5

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