Symposium Title: Novel Approach for the Development of Translational Biomarkers and Treatment Outcomes in Neurodevelopmental Disorders: Case Examples in Fragile X Syndrome and Related Disorders

Chair: Rebecca Shaffer, PsyD. Cincinnati Children's Hospital Medical Center

Discussant: David Hessl, PhD, University of California, Davis

Overview: Developing translational biomarkers in neurodevelopmental disorders with both known (e.g., Down Syndrome, Fragile X Syndrome) and unknown (e.g., idiopathic ASD) genetic causes is essential for advancing our understanding of pathophysiological mechanisms as well as for discovering novel treatments and stratification approaches for more individualized medicine. Despite the extensive and collaborative effort in the field to develop innovative behavioral and pharmacological interventions, we continue to be restricted by the limited clinical and biological relevance of available outcome measures. Each of the five presentations will address a different neuroscientific approach to examine clinically-relevant, quantifiable phenotypes in neurodevelopmental disorders, with Fragile X Syndrome (FXS) and Related Disorders being “case” examples. The goal of this symposium is to identify and discuss potential innovative methods for developing translational biomarkers in neurodevelopmental disorders more broadly. Though we highlight findings from FXS and related disorders, these translational approaches are usable across neurodevelopmental disorders and may be suitable for preclinical research as well. The first presentation examines expressive language and cognitive flexibility deficits in adolescents and adults with FXS to assess task-based functional connectivity using high-density EEG. The second presentation examines the evaluation of gaze aversive behavior in individuals with Fragile X Syndrome through emotional face eye tracking and the development of a highly innovative gaze aversion detection software. The third presentation examines the development of visual attention, utilizing a behavioral toy play task, and the use of heart rate activity to differentiate two etiologically distinct, genetically at-risk groups of infants based on ASD diagnostic outcomes in comparison to typically developing peers. The fourth presentation uses functional near infrared spectroscopy (fNIRS) with toddlers, including those with early language delays, to examine the relations between resting activity, functional brain activity, and language outcomes. The fifth presentation examines postural stability in aging individuals with Fragile X mental retardation 1 (FMR1) gene premutation during tests of posturography and their potential utility as a biobehavioral marker in monitoring the onset and progression of Fragile X related tremor and ataxia syndrome (FXTAS). Using different methods of assessing clinically-relevant phenotypes, these presentations highlight emerging approaches for developing novel biomarkers and treatment outcomes in neurodevelopmental disorders.

Paper 1 of 5

Title: Novel EEG Approaches to Examine Expressive Language and Cognitive Flexibility Deficits in Fragile X Syndrome

Authors: Lauren M. Schmitt¹, Jun Wang², Ernest Pedapati¹, Craig Erickson¹, John A Sweeney³

¹Division of Developmental and Behavioral Pediatrics, Cincinnati Children’s Hospital Medical Center; ²Zhejiang Normal University, China; ³Department of Psychiatry, University of Cincinnati

Introduction: Considerable progress has been made in establishing translational EEG biomarkers of Fragile X Syndrome (FXS), their modifiability via drug administration, and their molecular underpinnings. Though initial steps towards establishing the clinical significance of these neurophysiological alterations, linking them to parent reports of patient sensory hypersensitivities, little evidence yet links these neurophysiological alterations to direct measures of expressive language and cognitive function in this intellectual disability. The development of neurosciences strategies to study these clinically-relevant features is critical for our understanding of neurophysiological mechanisms underlying this and other neurodevelopmental disorders and development of novel treatments.
**Methods:** In a partially overlapping sample of full-mutation FXS and chronologically-aged and sex-matched typically-developing controls (TDC), participants completed an expressive language ('Talk/Listen') and/or a cognitive flexibility ('Reversal Learning') paradigm during continuous EEG recording using the 132-channel EEG system. During the Talk/Listen paradigm, participants (21 FXS, 20 TDC) repeated vocalizations of the phoneme “ah” every 1-2 seconds (s) for a total of 180 s, which were transmitted back to the participants in real-time ('Talk' Condition) and later passively listened to their recordings ('Listen' condition). Coherence between frontal and temporal cortices before and during speech was analyzed for both conditions and compared against naturalistic language measures. During the Reversal Learning paradigm, participants completed a two- and four-choice deterministic reversal learning task in which participants selected the stimulus that was in the correct location that switched at random after a variable number of correct responses. ERP and time-frequency analyses compared neural responses in the frontal and parietal cortices immediately following ‘Stay’ and ‘Reversal’ trials in each condition.

**Results:** During the Talk/Listen paradigm, individuals with FXS demonstrated reduced fronto-temporal connectivity in the left-hemisphere compared to TDCs, and this was related to a reduction in number of utterances during a naturalistic language task. Preliminary analysis demonstrate atypical frontal activity in FXS relative to TDC participants during Reversal trials, with some FXS patients showing exaggerated responses and other demonstrating suppressed responses. Clinical correlates of these different neural patterns are ongoing.

**Conclusion:** The development of speech is mediated feedforward/feedback processes by the Broca’s area within the inferior frontal gyrus in connection with the auditory cortex in order to compare it to the actual sound and make any necessary corrections for future speech sounds. Reductions in fronto-temporal coherence as observed in FXS suggest this process is disrupted in this population and may contribute to expressive language deficits. Moreover, exaggerated and dampened neural responses to feedback indicating a change in behavior is needed in FXS may reflect distinct mechanisms contributing to failures in flexibly shifting cognitive strategies necessary for adaptive behavior in this patient population. Overall, our findings indicate the feasibility of studying higher-level functions in neurodevelopmental populations with intellectual disability and each study implicates important targets for treatment and biomarker development in FXS and other neurodevelopmental disorders.

**References**


**Paper 2 of 5**

**Paper Title:** Utilizing a Biobehavioral Model of Visual Attention to Discriminate Outcomes in Infants at High Risk for Autism Spectrum Disorder

**Authors:** Debra Reisinger, Ph.D., Elizabeth Will, Ph.D., & Jane Roberts, Ph.D.

**Introduction:** Autism Spectrum Disorder (ASD) is highly prevalent with 1 in 59 children being identified each year. Infants with an older sibling diagnosed with autism (ASIBs) are at high genetic risk for developing ASD as are infants with fragile X syndrome (FXS). Impairments in visual attention have been identified as one of the earliest predictors of later social functioning relative to ASD in genetically high-risk infants. Additionally, studies have reported that children with ASD demonstrate elevated mean heart rate (HR) and less variability in HR than typically developing peers potentially implicating a mechanistic role in attention dysregulation. Given that a valid diagnosis of ASD rarely occurs prior to the age of two, efforts to identify early behaviors and biomarkers that are linked to outcomes are critical to the efforts of screening and intervening earlier. Further, it is ideal that infant trajectories are assessed rather than specific age points given early development can follow different, subtle patterns, occur at different ages, or be driven by different mechanisms. Utilizing longitudinal models to examine early development in
these high-risk infants, the present study examines visual attention trajectories across 9 to 24 months with infants with FXS and infant ASIBs in relation to typically developing peers based on their ASD outcomes. Furthermore, utilizing a subset of the present sample for exploratory analyses, we examine HR variability at 12 months and later ASD outcomes to initiate the use of heart activity as a biobehavioral mechanism of visual attention across two etiologically distinct groups infants at high-risk for ASD.

**Method:** Participants initially included 25 infant males with FXS, 23 infant males with an older sibling diagnosed with autism (ASIBs), and 26 typically developing (TD) infant males. The infants with FXS and infant ASIBs were parsed apart based on ASD outcomes at 3 years-of-age using a clinical best estimate (CBE) approach that included a team of expert clinicians who reviewed multiple measures including development, behavioral questionnaires, family/medical history, and the Autism Diagnostic Observation Schedule, Second Edition (ADOS-2). Implementation of the CBE process resulted in a total of five groups: infants with FXS (n=8), infants with FXS+ASD (n=17), infant ASIBs (n=15), infant ASIBs+ASD (n=8), and TD infants assessed at 9, 12, and 24 months of age. A toy play epoch from the Laboratory Temperament Assessment Battery (LabTAB) was used to measure the proportion of object attention toward a set of toy keys. Gaze behaviors were coded offline with a kappa of \( \geq 0.80 \) using Observer XT 10.5. Heart activity was also collected during the attention task and two variables were derived: mean HR and standard deviation (SD; variability) of HR.

**Results:** Piecewise multilevel models were utilized to examine object attention changes across 9 to 12 and 9 to 24 months of age across the 4 groups of participants in comparison to TD peers. Results of the model suggest infants with FXS (\( \beta=14.50, SE=8.27, t=1.75, p=0.08 \)) and infants with FXS+ASD (\( \beta=-14.25, SE=8.25, t=-1.73, p=0.08 \)) demonstrate marginally significantly changes overall across time in comparison to TD infants. Specifically, FXS+ASD (\( \beta=27.43, SE=10.99, t=2.49, p=0.01 \)) demonstrate significant increases in their object attention between 9 and 12 months in comparison to TD infants; whereas, infants with FXS (\( \beta=-35.69, SE=14.61, t=-2.44, p=0.02 \)) demonstrate significant declines in their object attention between 9 and 24 months in comparison to TD infants. Infant ASIBs and ASIBs+ASD did not demonstrate significant changes in their object attention between 9 and 12 or 9 and 24 months of age in comparison to their TD peers; however, visually, they demonstrate similar object attention patterns to the FXS and FXS+ASD infants across time based on their ASD outcomes. Two ANOVA’s were utilized to examine mean HR and SD of HR at 12 months across the five groups of infants based on their later ASD outcomes. For mean HR, no significant group differences were found, \( F(1,32)=0.78, p=0.55, \) partial \( \eta^2=0.09 \). Similarly, for SD of HR, no significant group differences were found, \( F(1,32)=1.09, p=0.38, \) partial \( \eta^2=0.12 \). While these results do not show statistical significance, the effect size estimates were moderate suggesting that these results may be underpowered. Descriptively, some interesting differences emerged within the subgroups. Specifically, at 12 months of age, infants with FXS+ASD (\( M=8.02, SD=1.18 \)) and infant ASIBs+ASD (\( M=7.05, SD=1.07 \)) exhibited similar HR variability to their TD peers (\( M=8.36, SD=0.73 \)); whereas, the infants with FXS (\( M=6.96, SD=1.07 \)) and infant ASIBs (\( M=5.97, SD=0.99 \)) exhibited less variability.

**Discussion:** Atypical object attention appears to emerge as early as nine months of age in infants at high genetic risk for ASD across two distinct etiologies. In FXS, atypical object attention patterns were observed and varied depending on their later ASD outcomes suggesting the added diagnosis of ASD in FXS may alter the development of their visual attention system across time. Similarly in ASIBs, object attention trajectories based on ASD outcomes are less clear statistically, but visually apparent and mirrored the infants with FXS and FXS+ASD suggesting the added ASD diagnosis may alter these two etiologically distinct groups similarly. When examining HR at 12 months, mean HR was not found to differ across groups; however, some interesting patterns emerged in HR variability. Unexpectedly, infants with FXS+ASD and ASIBs+ASD exhibited similar HR variability to their TD peers, but more variability than the infants with FXS and ASIBs without ASD potentially implying the heritable influences of ASD and relative physiological dysregulation in both high-risk groups at an early age. Ideally, early development should be examined longitudinally, like the visual attention data, in order to observe the small, but notable nuances of development that single time point data, like the HR data, may not reveal. Overall, both behavior and physiological markers of visual attention may differentiate these two distinct, genetically high-risk groups differently in infancy in relationship to their later ASD diagnostic outcomes.
References/Citations:


Paper 3 of 5

**Paper Title:** Gaze Tracking and Gaze Aversion Detection in a Study of Social Anxiety in Fragile X Syndrome

**Authors:** Michael Hong1,2, Lauren Schmitt3, Rebecca Shaffer2, Debra Reisenger2, Ernest Pedapati3, Craig Erickson2

1. University of Cincinnati College of Medicine
2. Division of Developmental and Behavioral Pediatrics, Cincinnati Children’s Hospital Medical Center

**Introduction:** Fragile X Syndrome (FXS) is caused by a CGG trinucleotide repeat within the FMR1 gene leading to methylation of the gene and loss of synthesis of FMRP. FXS is associated with developmental delay as well as attention problems, anxiety disorder, hyperactivity, and irritability. Gaze aversion is a well-known hallmark of FXS and recent social eye tracking findings have shown reduced eye gaze and increased pupillary response when viewing emotional faces. However, there remains ambiguity as to the exact mechanisms underlying social gaze abnormalities with inattention, social disinterest, and social anxiety as potential explanations.

**Methods:** Individuals with FXS (n=17) and age- and gender-matched TDC (n=17) and individuals with idiopathic ASD (n=17) completed emotional faces and social preference eye tracking tasks to evaluate gaze aversion and social interest, respectively. Participants completed a battery of cognitive testing and caregiver-report measures for neurobehavioral characterization. In a follow-up study, 36 individuals with FXS completed the same emotional faces eye tracking paradigm and a battery of
neuropsychological testing and caregiver-report measures. Gaze aversion detection software utilizing eye gaze and user camera facial recognition data with a variety of criteria was developed to assess gaze aversive behavior in FXS.

**Results:** Individuals with FXS exhibited reduced eye [F(4.12, 177.29) = 3.03, p = 0.021] and increased mouth gaze [F(4.38, 188.34) = 2.45, p = 0.046] to emotional faces compared to TDC. Gaze aversive findings were found to correlate with the Anxiety, Depression, and Mood Scale (ADAMS) Generalized Anxiety (r = -0.55, p = 0.032), ADAMS Manic/Hyperactivity (r = -0.59, p = 0.022), ABC-C Social Avoidance (r = -0.59, p = 0.028), and Social Communication Questionnaire (SCQ) (r = -0.55, p = 0.050). In the social interest task, while individuals with idiopathic ASD showed significantly less social preference, individuals with FXS displayed social preference similar to TDC [F(2, 46) = 3.40, p = 0.042]. Gaze aversion detection software supplemented with data manually coded by psychologists was able to successfully quantify gaze aversive behavior in individuals with FXS viewing emotional faces.

**Discussion:** Individuals with fragile X syndrome exhibit social deficits that may center more on social anxiety as opposed to the prominent reduction in social interest associated with autism spectrum disorder. Eye gaze findings alone were found to correlate with a range of psychological symptoms, including anxiety, hyperactivity, and behavioral problems. Techniques specifically detecting gaze aversive behavior in FXS offer the advantage to quantify FXS-specific symptoms for evaluating phenotype severity as well as use as an objective measure in intervention studies.

**References/Citations:**

**Paper Title:** Features of Resting State fNIRS Signal in Toddlers Predict Neural Activation to Language and Later Language Outcomes

**Authors:** Elizabeth Smith1,3, Afrouz Anderson1, Emma Condy3, Audrey Thurm4, Elizabeth Redcay5, Amir Gandjbakhche3

1. Division of Developmental and Behavioral Pediatrics, Cincinnati Children’s Hospital Medical Center
2. Human Development and Quantitative Methodology, University of Maryland
3. Section on Functional and Analytical Biophotonics, E
4. Office of the Clinical Director, National Institutes of Mental Health
5. Dept of Psychology, University of Maryland

**Introduction:** For toddlers with language delays, early detection and intervention are key to improvement and positive outcomes. However, considerable variability in language development exist both in the general population and in and other at risk groups, such as those with genetic risk, making detection of neural-based biomarkers for emerging language deficits important for improved early detection. Functional Near Infrared Spectroscopy (fNIRS) is a neuroimaging method that is highly tolerated by toddlers, including those with delays (Anderson et al., 2014), and is also less affected by the motion artifact inherent in this population compared to other neuroimaging techniques. Here, we measured features of the fNIRS signal during attentive rest and during perception of communicative cues (i.e., gestures and speech) in toddlers. The main goal of this study is to
determine the utility of measures taken at rest (which is associated with higher compliance) compared to those taken during presentation of controlled stimuli.

**Methods:** Toddlers (n=36, ages 2 and 3 years), including a small sample of toddlers with early language delays (n=8), wore a frontal fNIRS band while they watched short clips from popular children’s shows (attentive rest) and then again while they watched and listened to controlled stimuli relating to language and nonverbal communication. The controlled stimuli consisted of 4 conditions: Words, Nonwords, Gestures, and Nonmeaningful hand movements. fNIRS data from the attentive rest condition were used to derive an oxygenation variability index (OVI), essentially representing capacity for movement of oxygenated and deoxygenated blood, and lateralization index (LI), representing relative amounts of activity in the right versus left side of the frontal lobe. Relative changes in oxyhemoglobin were calculated for the 4 stimulus conditions. All children completed the Mullen Scales of Early Learning (MSEL), and 2-year-olds completed the MSEL again at age three-years.

**Results:** Oxyhemoglobin levels in left lateral cortex were higher for gesture versus speech and for nonwords versus words (t=2.21, p=.029; t= 2.77, p=.006). However, these patterns were not associated with the active rest measures (i.e., neither OVI nor LI). For two-year-olds, increased activation for gestures vs speech and meaningful vs nonmeaningful gestures in right medial cortex was associated with higher language scores at age 3 (t=2.73, p=.009; t=-2.49, p=.02). Both patterns of higher activation for gestures versus speech and for meaningful versus nonmeaningful gestures were significantly related to OVI (t=2.43, p=.023; t=-2.48, p=.025). Specifically, children with higher OVI during active rest at age 2 showed higher activation for gestures versus speech and for meaningful versus nonmeaningful gestures to a higher degree than those with lower OVI. These patterns were not significantly correlated with LI.

**Discussion:** Association of measures of neural activity during active rest (i.e, OVI) with functional activity and later outcomes is important for two reasons. First, association between OVI and functional activity (e.g., left lateral frontal activity for language tasks) supports validity of the OVI, showing that meaningful individual differences in oxygenation variability can be measured. Second, association between OVI and the brain patterns related to better language outcomes at age 3 indicates potential use of resting state metrics, which are generally easier to obtain than metrics for neural activity to controlled stimuli, in early detection of risk for later language delays.

**References:**

**Paper Title:** Static and Dynamic Postural Control Deficits in Aging Fragile X Mental Retardation 1 (FMR1) Gene Premutation Carriers

**Authors:** Zheng Wang1*, Pravin Khemani2, Lauren M. Schmitt3, Su Lui4, Matthew W. Mosconi5,7
1. Department of Occupational Therapy, University of Florida, Gainesville, FL 32611, USA
2. Department of Neurology, Swedish Neuroscience Institute, Seattle, WA 98121, USA
3. Division of Developmental and Behavioral Pediatrics, Cincinnati Children’s Hospital Medical Center, Cincinnati, OH 45229, USA
4. Huaxi Magnetic Resonance Research Center (HMRRC), Department of Radiology, West China Hospital of Sichuan University, Sichuan, Chengdu 610041, China
5. Schieffelbusch Institute for Life Span Studies, University of Kansas, Lawrence, KS 66045, USA
6. Clinical Child Psychology Program, University of Kansas, Lawrence, KS 66045, USA
7. Kansas Center for Autism Research and Training (K-CART), University of Kansas, Lawrence, KS 66045, USA

**Introduction:** Individuals with premutation alleles of the fragile X mental retardation 1 (FMR1) gene are at risk of developing fragile X associated tremor/ataxia syndrome (FXTAS) during aging (Hall & O'Keefe, 2012; Jacquemont et al., 2003). Characterization of motor issues associated with aging in FMR1 premutation carriers is needed to determine neurodegenerative processes and establish new biobehavioral indicators to help identify individuals at greatest risk of developing FXTAS.

**Methods:** We examined postural stability in 18 premutation carriers ages 46-77 years and 14 age-matched healthy controls. No premutation carrier reported any clinical concerns at study entry. Participants completed a test of static stance and two tests of dynamic stances on an AMTI force platform in which they continuously swayed anterior-posteriorly (AP) or mediolaterally (ML). The cytosine-guanine-guanine (CGG) repeat length was measured for each premutation carrier, and clinical neurological and MRI evaluations were conducted to identify carriers who currently met criteria for FXTAS.

**Results:** Compared to controls, carriers showed increased center of pressure (COP) variability in the ML (COPML) direction during static stance and reduced COPAP variability during dynamic AP sway. They also showed reductions in COPML complexity during each postural condition. Increased sway variability during static stance and decreased sway variability in target directions during dynamic sways were associated with greater CGG repeat length and more severe clinically-rated posture and gait abnormalities. Of the 14 premutation carriers who completed both the clinical neurological and MRI exams, 7 met published criteria (Jacquemont et al., 2003) for definite/probable FXTAS (FXTAS+), whereas 7 currently showed no clinical or MRI signs of FXTAS (FXTAS-). FXTAS+ individuals showed reduced COPAP variability compared to FXTAS- carriers and healthy controls during dynamic AP sway.

**Discussion:** Our findings indicate that aging FMR1 gene premutation carriers show static and dynamic postural control deficits relative to healthy controls implicating degenerative processes of spinocerebellar and cerebellar-brainstem circuits that may be independent of or precede the onset of FXTAS. Our finding that FXTAS+ and FXTAS- premutation carriers differed on their level of intentional AP sway suggests that neural control of dynamic postural control may be differentially impacted in patients with FXTAS, and its measurement may be useful for rapidly and precisely identifying disease presence and onset.

**References:**