Symposium Title: The FMR1 Premutation: Biomarkers and Discriminating Phenotypes

Chair: Jessica Klusek¹

Discussant: Kim Cornish²

Overview: The FMR1 premutation is a common genetic mutation, affecting 1:151 females and 1:468 males, that is associated with risk for having a child affected by fragile X syndrome, the leading form of inherited intellectual disability. While “carriers” of the FMR1 premutation were once mistakenly believed to be asymptomatic, it is now clear that the FMR1 premutation confers risk for a range of mental and physical health conditions that reduce quality of life for these individuals and their families. Yet, useful biomarkers that can predict clinical risk in this population have not been identified, hindering detection, prevention, and treatment efforts. This symposium features an interdisciplinary, multi-institute panel who will discuss new research on the phenotypic presentation of the FMR1 premutation and associated molecular and physiological biomarkers.

Paper 1 of 3

Paper Title: Autonomic Dysfunction is Associated with Pragmatic Language Impairments in Women with the FMR1 Premutation and the Broad Autism Phenotype

Authors: Jessica Klusek¹, Jane E. Roberts³

Introduction: Emerging work suggests that impairments in pragmatic language (i.e., social language) are seen at increased rates among women with the FMR1 premutation and are associated with negative language outcomes for their children with fragile X syndrome (Klusek et al., 2016; Losh et al., 2012). Yet, the mechanisms underlying pragmatic language impairments in the FMR1 premutation have not been identified, limiting targeted treatment and understanding of relevant gene-brain-behavior pathways. Autonomic nervous system dysfunction is a primary candidate mechanism for pragmatic language impairments in the FMR1 premutation, as autonomic dysfunction is a hallmark feature of the fragile X full mutation that is believed to impact social engagement. The present study had two objectives. First, we sought to replicate earlier reports of pragmatic deficits in the FMR1 premutation through comparison of pragmatic language ability across new, independent samples of mothers with the FMR1 premutation and control mothers of typically developing children. We also included a comparison group of mothers of children with autism, who are at risk for pragmatic language deficits as part of the broad autism phenotype and can inform disassociation across phenotypes. Secondly, we investigated autonomic dysregulation as a potential mechanism for pragmatic deficits by examining the relationship between cardiac indicators of autonomic function and pragmatic language ability across groups.

Methods: Participants included 43 mothers with the FMR1 premutation, 27 control mothers of typically developing children, and 24 mothers of children with autism. The groups did not differ on age, IQ, race, or education level (p’s < .177). Cardiac activity was sampled in a 3-min baseline condition and mean estimates for heart rate (an index of general arousal level) and respiratory sinus arrhythmia (an index of parasympathetic “rest and restore” autonomic function) were derived. Pragmatic language violations were coded from a 20-min conversational interview using a modified version of the Pragmatic Rating Scale (Losh et al., 2012; Klusek et al., 2016). Samples were coded by two independent raters and consensus scores were derived, with reliability prior to consensus at ICC(3,2)=.74. Group differences in pragmatic language were tested using a general linear regression model with group as a predictor and planned post-hoc comparisons with Tukey’s adjustment for multiple comparisons. Then, a series of general linear models tested each of the cardiac variables, group, and their interaction as predictors of pragmatic language.

¹ Department of Communication Sciences and Disorders, University of South Carolina
² Monash Institute for Cognitive and Clinical Neurosciences, Monash University, Melbourne
³ Department of Psychology, University of South Carolina
Results: Mothers with the FMR1 premutation and mothers of children with autism committed significantly more pragmatic language violations than control mothers (p’s <.008), whereas pragmatic language ability did not differ between the FMR1 premutation and ASD mother groups (p =.328). Respiratory sinus arrhythmia was a significant predictor of pragmatic language ability (p <.001), with the main effect of group and its interaction not accounting for significant variance in the model (p’s >.886).

Discussion: This study provides further support for pragmatic language deficits as a component feature of the FMR1 premutation phenotype. We also replicated earlier work suggesting shared pragmatic phenotypes across the FMR1 premutation and the broad autism phenotype (i.e., Losh et al., 2012), which may suggest a role of FMR1 in features associated with autism and the broad autism phenotype. Reduced parasympathetic control was associated with pragmatic language deficits across all groups, implicating autonomic dysregulation in pragmatic language variation relevant to both typical and atypical populations. This finding sheds light on physiological underpinnings of behavior and informs mechanistic targets that may be useful for pharmacological/behavioral treatment studies for autism and fragile X-associated conditions.

References/Citations:

Paper 2 of 3

Paper Title: Automated Screening for Fragile X Premutation Carriers Based on Linguistic and Cognitive Computational Phenotypes

Authors: Arezoo Movaghar4 5 6, Jan Greenberg4 7, Marsha Mailick4, Audra Sterling4 8, Krishanu Saha2 3 4

Introduction: The fragile X mental retardation 1 (FMR1) gene is responsible for healthy brain development and functioning, and the full mutation of FMR1 (CGG repeats >200) causes fragile X syndrome (FXS). While individuals with elevated number of CGG repeats (55-200), called FX premutation carriers, do not have FXS, they are at a higher risk for health difficulties as well as for having a child with a disability. Considering the prevalence of the FMR1 premutation (1 in every 151 females and 1 in every 468 males), early detection of FMR1 expansions has a significant importance for public health (Mailick et al., 2014). Genotyping broad populations to identify individuals with a gene abnormality is a significant challenge, because the process is resource- and time-intensive and may require specialists to interpret test results. Therefore there is a need to develop novel, efficient methods to rapidly pre-screen the population for FMR1 status without the broad use of genetic tests.

Methods: Computational phenotyping enables researchers to discover new phenotypic patterns from electronic health records and clinical data. In a novel approach to phenotyping, we have utilized five-minute speech samples (Sterling et al., 2013) and cognitive assessments (measured by the BRIEF-A standards) collected via either in-person or over the phone interviews from 200 females, ages 25 to 79 years. As a part of phenotype discovery, comprehensive linguistic and cognitive profiles of FX premutation carriers were compared with profiles of mothers of children with autism spectrum disorders as the comparison group. Using

---

4 Waisman Center, University of Wisconsin-Madison
5 Wisconsin Institute for Discovery, University of Wisconsin-Madison
6 Department of Biomedical Engineering, University of Wisconsin-Madison
7 Department of Social Work, University of Wisconsin-Madison
8 Department of Communication Sciences and Disorders, University of Wisconsin-Madison
feature selection module, an optimized profile was created to represent the linguistic and cognitive phenotypes of FX premutation carriers. This profile was used to develop a data-driven classifier to identify *FMR1* premutation status in new samples.

**Results:** The results of computational phenotyping indicated that FX premutation carriers have significant impairment in cognitive functioning including the ability to initiate an activity, organize materials, and complete successful self-evaluation. Significant elevation in linguistic dysfluencies was observed in these females. By combining the linguistic and cognitive measures in a random forest classifier, FX premutation carriers were discriminated automatically from control group with 82% accuracy (0.82 F1 score) with an estimated four-fold decrease in the screening costs.

**Discussion:** The present study highlights an efficient method, which could be used for screening broad populations to identify individuals with possible *FMR1* gene expansions prior to genetic test. Collecting phenotypic data such as linguistic and cognitive features are simple and convenient for both examiners and participants and will significantly reduce resources required for screening, especially if data collection is implemented on a mobile device (e.g., via Apple’s ResearchKit). Language and cognitive deficits occur in a variety of clinical syndromes such as Alzheimer’s and Parkinson’s disease. Therefore the proposed framework potentially can be customized for phenotyping in these other neurological disorders.

**References/Citations:**

**Paper 3 of 3**

**Paper Title:** Identifying Modifying Genes to Explain the Variation in Severity of *FMR1* Premutation-Associated Disorders

**Authors:** Emily G. Allen⁹, Krista Charen⁹, Lisa Shubeck⁹, Christina Trevino⁹, David Cutler⁹, Michael Zwick⁹ ¹⁰, Michael Epstein⁹, Peng Jin⁹, Stephen T. Warren⁹, Stephanie L. Sherman⁹

**Introduction:** *FMR1* premutation-associated disorders are well established as clinically significant, placing a medical burden on the individual and their family. Fragile X-associated primary ovarian insufficiency (FXPOI) is a condition characterized by symptoms of early menopause and infertility and affects about 20% of women with the premutation (PM; 55-200 repeats in the 5’UTR of the X-linked *FMR1* gene). Fragile X-associated tremor/ataxia syndrome (FXTAS) is a neurodegenerative disorder that leads to late-onset characterized by tremor, ataxia and cognitive decline in about 40% of men and 15% of women with the PM. Although both disorders are clinically significant, not all carriers experience the disorder, and among those who do, the range of severity is broad. We hypothesize that there are other genetic variants, in addition to the PM repeat number, that modify the age of onset and severity of FXPOI and of FXTAS.

**Methods:** To test our hypothesis, we are currently conducting a whole genome sequencing (WGS) study among PM carriers whose onset of symptoms is experienced in the tails of the age distribution. For FXPOI, we compare women with the PM who have onset of FXPOI diagnosed before age 35 (cases) with those who had menopause after age 50 (controls). For FXTAS, we compare men and women with the PM who have onset of obvious motor symptoms prior to age 65 years (cases) with men who had no motor symptoms prior to age 70 years (controls). We first prioritize identified sequence variants from the WGS using a well-established pipeline. From these data, we will take the top candidate genes and screen them using *Drosophila* models as a high-throughput, whole organism functional screen. Models will include the knock-out (KO) of the candidate gene and the KO on

⁹ Department of Human Genetics, Emory University
¹⁰ Department of Pediatrics, Emory University
a PM background (90 CGG repeats). Using both models will allow us to determine whether there are modifying effects of the candidate gene specifically with the PM.

**Results:** To date, we have recruited 74 women carrying the PM with early-onset FXPOI and 59 women with age of menopause >50 yrs. For FXTAS, we have recruited 69 individuals (primarily men) with early onset FXTAS and 27 men without motor symptoms by age 70 years. Recruitment will continue until we reach the target sample sizes of 100 cases and 100 controls for each disorder. Although recruitment for the FXPOI study is straightforward, recruitment for controls with the FXTAS project is proving to be difficult. WGS has been successful using either blood or saliva samples, easing the burden of participation on the family. As our current sample sizes for the FXPOI study are larger, we will present the preliminary results from the WGS and *Drosophila* model system.

**Discussion:** The overall goal of this project is to elucidate novel molecular mechanisms for PM-associated disorders to facilitate early risk assessment and potentially identify treatment targets. Also, genetic variants identified as modifiers of these disorders on a PM background may play an important role in idiopathic primary ovarian insufficiency or neurodegenerative disorders.