



# Vagal Tone as a Putative Mechanism for Pragmatic Competence: An Investigation of Carriers of the *FMR1* Premutation

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## Abstract

Pragmatic language skills exist across a continuum in typical and clinical populations, and are impaired in many neurodevelopmental disorders, most notably autism. The mechanisms underlying pragmatic impairment are poorly understood, although theory suggests dampened vagal tone plays a role. This study investigated the *FMR1* premutation as a genetic model that may lend insight into the relationship between vagal function and pragmatic ability. Participants included 38 women with the *FMR1* premutation and 23 controls. Vagal tone accounted for significant variance in pragmatics across both groups and statistically mediated the effect of *FMR1* premutation status on pragmatic ability. Results support vagal tone as a biophysiological correlate of pragmatic ability, which informs potential mechanistic underpinnings and could have implications for targeted treatment.

**Keywords** Fragile X carrier · HRV · RSA · Social communication · Social (Pragmatic) Communication Disorder

The social use of language, or pragmatic language, is a fundamental component of communicative competence (Prutting 1982). Pragmatic language skills encompass knowing what to say, and how and when to say it; this knowledge is implicitly required for successful communication, such as holding a back-and-forth conversation, telling a cohesive story, and clarifying misunderstandings. Deficits in pragmatic language can have a substantial impact on social functioning and are linked with loneliness, poor social-emotional adjustment, difficulty managing social relationships, social isolation, externalizing problems, antisocial behavior, and risk for psychological disorders such as social phobia (Coplan and Weeks 2009; Geurts et al. 2004; Jobe and White 2007; Ketelaars et al. 2010; Laws et al. 2012; Whitehouse et al. 2009). Despite evidence that the majority of individuals with pragmatic difficulties in childhood continue to face persistent problems into adulthood (Conti-Ramsden et al.

2001; Whitehouse et al. 2009), there is a scarcity of research on pragmatic competence in adults.

Many neurodevelopmental disorders, most notably autism spectrum disorder (ASD), are characterized by pragmatic deficits. Impairment in pragmatic aspects of communication is a feature that is universally observed across all individuals with ASD (Landa 2000). Indeed, the DSM-5 diagnostic criteria for ASD relies heavily on the presence of features that fall within the domain of pragmatic competence, such as failure to engage in back-and-forth conversation, poor integration of verbal and nonverbal communication, reduced initiation and sharing of interests, lack of gestures or facial expressions, stereotyped or repetitive speech, topic pre-occupations, and abnormal eye contact (American Psychiatric Association 2013). Pragmatic deficits are also central to broader autism phenotypes observed in unaffected relatives of individuals with ASD, suggesting that pragmatic impairment is a core, biologically mediated component of ASD that is linked to underlying genetic susceptibility (Losh et al. 2008; Piven et al. 1997).

Pragmatic impairment in individuals who do not meet diagnostic criteria for ASD has also been frequently described in the literature, accompanied by much debate as to whether these deficits represent a distinct disorder or a milder form of ASD (e.g., Bishop and Norbury 2002; Brook and Bowler 1992; Reisinger et al. 2011). Recently,

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in recognition that pragmatic impairment can occur independently of ASD, Social (Pragmatic) Communication Disorder (SCD) was added as a new diagnostic category in the DSM-5 (American Psychiatric Association 2013). The introduction of SCD as a distinct disorder highlights that pragmatic deficits exist across a continuum in typical and clinical populations and provides a common framework from which to refine the conceptualization of pragmatic deficits occurring within and without of the context of ASD (Swineford et al. 2014). Understanding the presentation of pragmatic difficulties observed across typical and at-risk groups is important because it contributes to open questions in the field regarding the continuum of ASD-related features within the general population, and the mechanistic specificity of such features. The present study investigated biophysiological correlates of pragmatic competence occurring across neurotypical women and women who are at genetic risk for pragmatic language deficits due to carrying a genetic abnormality on the *FMR1* gene.

### The Role of the Vagal Nerve in Pragmatic Competence

According to Polyvagal Theory (Porges 2001, 2007), individual differences in social engagement skills can be accounted for by autonomic regulation via the vagal nerve. The vagus provides parasympathetic input that calms the heart, stifling sympathetic “fight or flight” impulses and allowing the body to establish a controlled physiological state that facilitates social engagement. Polyvagal Theory posits that the vagal nerve evolved in mammals to include myelinated fibers that allow parasympathetic signals to travel to the heart more efficiently, thereby promoting adaptive social behavior. In support of Polyvagal Theory, a wealth of evidence shows that high vagal tone is linked with enhanced social functioning in both children and adults (e.g., Calkins and Keane 2004; Demaree et al. 2004; Kok and Fredrickson 2010; Quintana et al. 2012). Applying Polyvagal Theory specifically to the study of pragmatic behavior, high vagal tone is associated with better concurrent pragmatic skills in children with ASD (Klusek et al. 2013) as well as at a one-year follow-up (Watson et al. 2010). Knowledge of vagal-pragmatic associations is limited to these two reports, and it remains unclear whether these relationships generalize to other clinical groups. Importantly, Polyvagal Theory provides a biophysiological explanation for pragmatic competence that can be applied across the continuum of typical and atypical development, which is consistent with recent scientific initiatives favoring dimensional, transdiagnostic approaches to understanding the biological bases of behavior (e.g., the Research Domain Criteria (RDoC); Insel 2014). In this study we investigated the *FMR1* premutation—a genetic

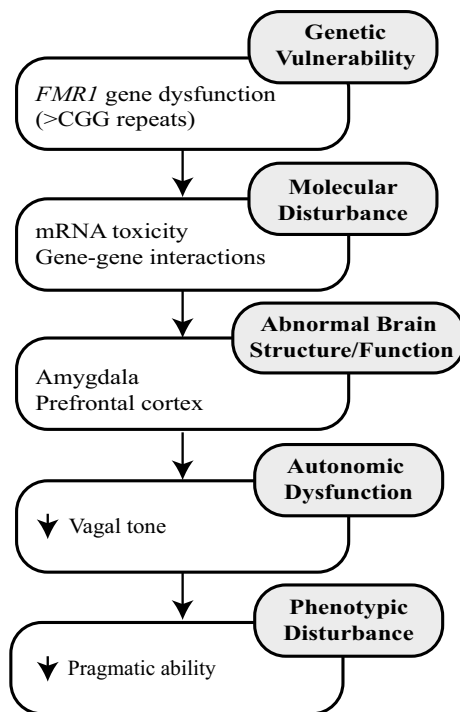
abnormality associated with pragmatics deficits and dampened vagal tone—as a model for understanding the link between vagal function and pragmatic ability.

### The *FMR1* Premutation

The *FMR1* premutation affects 1 in 151 women and occurs when the trinucleotide (CGG) sequence on the *Fragile X Mental Retardation-1* (*FMR1*) gene expands to 55–200 repeats (Maddalena et al. 2001; Seltzer et al. 2012). This genetic abnormality is associated with excess production of *FMR1* messenger RNA (mRNA), which is thought to disrupt numerous cellular pathways, causing neuronal damage and death (Hagerman and Hagerman 2013). It was once mistakenly believed that carriers of the *FMR1* premutation showed no associated clinical effects besides the risk of passing the mutated gene to their children, which may result in fragile X syndrome. However, it is now evident that the *FMR1* premutation is associated with a range of physical, cognitive, and affective symptoms and disorders that impact health and quality of life (see Wheeler et al. 2017 for review). Notably, the penetrance of associated clinical effects is incomplete and the lack of identified biomarkers limits identification, prevention, and treatment efforts for this heterogeneous group.

A variety of social difficulties have been documented as part of the *FMR1* premutation phenotype, such as atypical use of eye gaze, elevated broad autism phenotype traits, and ASD occurring in about 5% of females with the premutation (Clifford et al. 2007; Klusek et al. 2017b, c; Losh et al. 2012; Schneider et al. 2016). Social deficits in this group extend to the domain of pragmatics. During conversational interaction with an examiner, women with the *FMR1* premutation exhibit higher rates of pragmatic violations relative to control women, such as topic perseveration, interrupting, and over-talkativeness (Losh et al. 2012). Pragmatic violations in women with the *FMR1* premutation are associated with poorer language outcomes in their children with fragile X syndrome, demonstrating the clinical significance of these features for both the individual and their family (Klusek et al. 2016). Pragmatic deficits are also seen in individuals with the full mutation on *FMR1* (i.e., fragile X syndrome), with some evidence that pragmatic deficits are tied to variation at the *FMR1* locus (Klusek et al. 2014; Losh et al. 2012). Thus, pragmatic impairment represents a central phenotype associated with *FMR1* gene dysfunction.

*FMR1*-associated conditions provide a unique genetic context from which to study the clinical impact of vagal dysregulation. Dampened vagal tone is a well-documented, hallmark feature of fragile X syndrome that emerges early in the developmental course and is believed to reflect a biological signature of *FMR1* gene dysfunction (see Klusek et al.



**Fig. 1** Conceptual framework of atypical vagal function in the *FMR1* premutation. Framework based on the conceptual work of Roberts et al. (2011)

2015, for review; Roberts et al. 2012). Recent evidence suggests that dampened vagal tone also extends to the *FMR1* premutation, and is associated with *FMR1* molecular genetic indices. Klusek et al. (2017a) detected lower baseline vagal tone in women with the *FMR1* premutation compared to neurotypical control women, after accounting for confounds such as age, stress, and medication use. Interestingly, a significant, moderate association was detected between higher vagal tone and higher *FMR1* mRNA expression, which was unexpected given that excess *FMR1* mRNA production is believed to be toxic to the neural system (Hagerman and Hagerman 2013). Thus, some questions remain regarding the specific mechanisms by which *FMR1* is linked to vagal function; however, this body of research, overall, suggests that vagal tone may represent an etiologically meaningful marker in *FMR1*-associated conditions. Combining this evidence with Polyvagal Theory, we hypothesized that vagal tone may mediate the relationship between *FMR1* gene dysfunction and clinical outcomes. Figure 1 presents a conceptual framework describing the process by which vagal dysregulation is thought to relate to pragmatic language difficulties, using the *FMR1* premutation as a model. In this framework, altered *FMR1* gene function leads to disturbance in cortical structures and pathways, particularly in the areas of the amygdala and prefrontal cortex (Brown and Stanfield 2015; Hessl et al. 2007, 2011). These brain regions are

also thought to be important for autonomic regulation (see Thayer et al. 2012). Disturbance in these higher brain circuits is believed to result in impaired ability to process and respond to input regarding the body's physiological state via the vagal nerve (the vagus provides bi-directional communication between the brain and heart via projections to the brainstem and sinoatrial node; Porges 2003). Thus, capability for quick, flexible physiological adjustments via the vagal nerve is reduced, which, consistent with Polyvagal Theory, negatively impacts social engagement, including pragmatic competence. In line with this conceptual framework, the present study employed statistical mediation analysis to test the hypothesis that vagal tone mediates the impact of *FMR1* premutation genetic status on pragmatic language ability. By leveraging the *FMR1* premutation as an etiological model for pragmatic deficits, this study aims to yield novel information on the physiological correlates of social communication behavior that may have relevance to both individuals with and without *FMR1* mutations.

## Methods

### Participants

Participants included 38 women with the *FMR1* premutation and 23 neurotypical control women. Participants were enrolled in a larger study focused on women with the *FMR1* premutation, which has been previously described (Klusek et al. 2017a). All participants were native speakers of American English and biological mothers of a child 4 years or older. The *FMR1* premutation was confirmed via genetic testing (87%) or medical record review. All control women were mothers of typically developing children who had not been diagnosed or treated for any developmental delays or disorders, and 70% of controls completed genetic testing to rule out the *FMR1* premutation through dual enrollment in a related pilot study.

Women with the *FMR1* premutation were recruited through word of mouth and social media targeting local families, or through their children who were participating in developmental studies of fragile X syndrome with nation-wide recruitment. Control women were recruited locally through flyers posted at pediatrician offices, word of mouth, and social media. The sample was primarily Caucasian (92% of the *FMR1* premutation group; 86% of controls). The groups did not differ on age, IQ, or education level. Chronic stress can influence the integrity of physiological regulation systems (McEwen 1998), so information on parenting stress was collected and tested as a potential confound; as expected, the *FMR1* premutation group reported higher levels of parenting stress (see Table 1 for demographic information).

**Table 1** Group characteristics

Variable	Group		Test of group differences ( <i>p</i> -value)
	<i>FMRI</i> premutation ( <i>n</i> =38)	Control ( <i>n</i> =23)	
Age in years			
<i>M</i> ( <i>SD</i> )	44.51 (8.61)	42.16 (7.99)	0.293
Range	25.53–59.96	33.10–64.02	
IQ <sup>a</sup>			
<i>M</i> ( <i>SD</i> )	105.35 (12.90)	106.26 (11.03)	0.800
Range	81.00–130.00	83.00–135.00	
Education level (%)			
High school or lower	47	26	0.221
Bachelor's degree	29	30	
Master's degree	24	26	
Professional degree	0	17	
Medication use (%)			
Atypical antipsychotics	3	0	0.018*
Classical antipsychotics	3	0	
Antidepressants	36	0	
Mood stabilizers	6	0	
Stimulants	3	4	
Antianxiety	0	0	
Anticonvulsants	3	0	
Parenting stress percentile <sup>b</sup>			
<i>M</i> ( <i>SD</i> )	58.39 (22.33)	36.82 (23.44)	0.001*
Range	4.00–96.00	1.00–82.00	

\**p* < 0.05<sup>a</sup>Measured with the Composite IQ score of Kaufmann Brief Intelligence Test-II (Kaufman and Kaufman 2004)<sup>b</sup>Measured with the Total Stress percentile of the Parenting Stress Inventory-4 (Abidin 2013)

Information on medication use was collected given that certain psychotropic medication can influence cardiac activity, although the direction and magnitude of the relationship is complex and varies across individuals (O'Brien and Oyebo 2003). In a recent systematic review and meta-analysis of psychotropic medications, only tricyclic antidepressants and clozapine were found to have a statistically significantly influence heart rate variability (Alvares et al. 2016); none of the participants in the present sample were taking either clozapine or tricyclic antidepressants. More women with the *FMRI* premutation than control women used one or more psychotropic medications (42% vs. 13%); psychotropic medication use was covaried in analyses. Information on the use of other cardioactive medications (e.g., beta blockers) was also collected. Two control participants reporting using an antihypertensive medication. Results were the same when these participants were excluded, so these participants were retained in analyses.

## Procedure

Participants provided informed consent and all procedures were approved by the Institutional Review Board of the University of South Carolina. Baseline vagal tone was sampled after consent was obtained and before the initiation of other assessment activities. The conversational sample was collected approximately an hour into the research protocol.

## Measures

### Vagal Tone

Baseline vagal tone was measured from a 3-min resting condition in which participants viewed a video of an ocean scene. An Actiwave Cardio monitor (CamNtech Ltd., Cambridge,

UK) sampled the ECG signal at 1024 Hz via two electrodes placed on the participant's chest. The IBI series was extracted using QRSTool (Allen et al. 2007). Artifacts and arrhythmias were edited with CardioEdit software (Porges 1985; Porges and Bohrer 1990), with all files requiring <5% correction. Mean respiratory sinus arrhythmia (RSA), an index of vagal tone, was extracted using CardioBatch (Porges and Bohrer 1990) with a 0.12–0.40 Hz bandpass filter.

## Pragmatic Language

Participants engaged in a 20 min conversational interaction with an examiner that was guided by a series of probe questions intended to elicit conversation on familiar, shared experiences (e.g., “What did you enjoy most during your time in high school?”). To ensure opportunities for conversational back-and-forth, examiners were trained to comment, offer information, and ask follow-up questions. The samples were videotaped and coded off-line for pragmatic violations using a modified version of the Pragmatic Rating Scale (Landa et al. 1992), which has been used to capture pragmatic variation in non-disordered adults (see Klusek et al. 2014; Losh et al. 2012). The scale captures 26 possible pragmatic language violations that are coded on a scale of 0–2 based on operational definitions of the frequency or severity of each violation. A total score is computed by summing individual items, with a higher score reflecting greater pragmatic difficulty. Each videotaped sample was coded by two independent raters and consensus scores were produced through discussion. Rater 1 was unable to remain blind to group membership due to her role in participant assessment; rater 2 was blind to the group membership of all participants. Prior to consensus, inter-rater reliability was ICC (3, 2)=0.72, which is considered “good” agreement (Cicchetti 2001).

## Results

### Descriptive Statistics and Preliminary Analyses

Descriptive statistics were computed (see Table 2) and the data were examined for normality, with no corrections

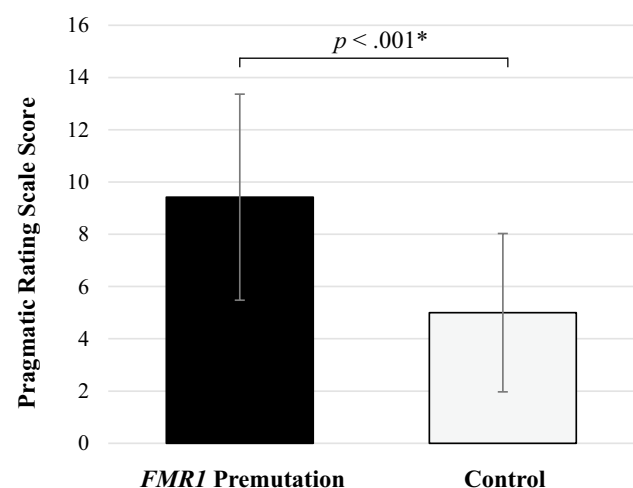
**Table 2** Descriptive statistics

Variable	Group	
	<i>FMRI</i> premutation	Control
Vagal tone		
<i>M</i> ( <i>SD</i> )	4.90 (1.32)	5.69 (1.00)
Range	1.78–7.64	2.88–7.26
Pragmatic Rating Scale score		
<i>M</i> ( <i>SD</i> )	9.42 (3.94)	5.00 (3.03)
Range	2.00–17.00	0–11.00

necessary. Pearson correlations tested potential confounds to inform the final statistical models. No associations were detected between parenting stress and vagal tone within or across the groups (all  $r$ 's < .09, all  $p$ 's > 0.629). Parenting stress level was also not associated with pragmatic skills within or across groups (all  $r$ 's < .21, all  $p$ 's > 0.253). Within the groups, there were no differences between the vagal tone of individuals who were or were not using medication (all  $p$ 's > 0.304); a trend for lower vagal tone in medicated individuals was detected when the groups were collapsed ( $p = 0.067$ ). Based on these preliminary analyses, psychotropic medication use (coded as present or absent) was integrated as a covariate in the models including vagal tone, to control for psychotropic medication use as a potential confounder of results. We did not control for parenting stress level in the final models given that preliminary analyses did not support an association. The groups differed on mean vagal tone estimates, with reduced vagal tone in the *FMRI* premutation group relative to controls ( $p = 0.016$ ,  $\eta_p^2 = 0.09$ ).

### Group Differences in Pragmatic Ability

A general linear model tested group as a predictor of the Pragmatic Rating Scale score. Pragmatic language difficulties were elevated in women with the *FMRI* premutation ( $F [1, 59] = 21.26$ ,  $p < 0.001$ ), with partial eta squared ( $\eta_p^2$ ) of 0.27 consistent with a “large” effect size (Cohen 1988). Group differences are presented in Fig. 2.



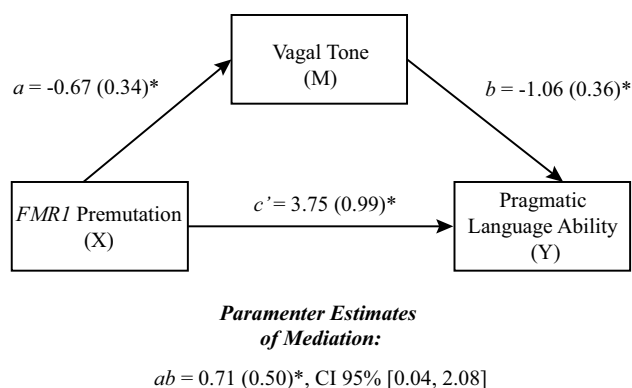
**Fig. 2** Group differences in pragmatic language competence. A higher score on the Pragmatic Rating Scale indicates increased pragmatic language difficulties

### The Indirect Effect of *FMR1* Premutation Group Membership on Pragmatics via Vagal Tone

Prior to conducting statistical mediation analysis, we confirmed that there was no interaction between the independent variable (group membership) and the mediator variable (vagal tone), which is a statistical assumption of mediation models (MacKinnon et al. 2007). To test this assumption, we fit a general linear model including group, vagal tone, and their interaction as predictors of pragmatic ability, with medication use included as a covariate. A main effect for vagal tone was detected, where higher vagal tone was associated with increased pragmatic competence ( $F [1, 56] = 4.83, p = 0.032, \eta_p^2 = 0.08$ ). The group-by-vagal tone interaction term was not significant ( $F [1, 56] = 0.28, p = 0.597, \eta_p^2 < 0.01$ ), indicating that the association between vagal tone and pragmatics did not differ according to group. In this model, the main effects for group ( $F [1, 56] = 1.63, p = 0.201, \eta_p^2 = 0.03$ ) and medication use ( $F [1, 56] = 0.36, p = 0.550, \eta_p^2 = 0.01$ ) were also not significant.

Next, a formal statistical test of mediation evaluated the putative pathway where the *FMR1* premutation genetic status impacts pragmatic ability via dampened vagal tone. Mediation analyses were conducted using the product of coefficients approach with bias-corrected bootstrapping for significance testing, as research supports that this test of mediation as more powerful relative to other options (Fritz and MacKinnon 2007) and does not make assumptions about the shape of the sampling distribution of the indirect effect (MacKinnon et al. 2004; Shrout and Bolger 2002). Further, the approach has been shown to coincide with causal inference approaches to mediation when there is no interaction of the independent and mediator variables, as demonstrated here, and the outcome is continuous (Valeri and VanderWeele 2013). In the product of coefficients approach, the presence of a mediating mechanism is established through assessing statistical significance of the indirect effect of the independent variable on the dependent variable through the mediator variable, using path tracing rules introduced in the early structural equation modeling literature (see Fairchild and McDaniel 2017, for review).

Analyses were conducted with the PROCESSv2.16 macro (Hayes 2012) for IBM SPSS Statistics (SPSS 2012). Although a limitation of PROCESS can be its use of listwise deletion to handle missing data, this was not a concern for our dataset, which had complete data for all participants. A single mediator model was specified to test the indirect effect of group membership on pragmatic ability via vagal tone, controlling for medication use. A 95% confidence interval was calculated for the indirect effect using 5000 bootstrap samples. Results yielded a significant indirect effect point estimate of  $ab = 0.71$  with a corresponding 95% bias-corrected, bootstrapped confidence interval equal to [0.04,



**Fig. 3** The indirect effect of *FMR1* premutation status on pragmatic language ability through vagal tone. Parameter estimates are controlling for medication use. *X* independent variable, *M* mediator, *Y* dependent variable. *ab* represents the indirect effect of *X* on *Y* through the mediating variable.  $*p < 0.05$

**Table 3** Correlations with CGG repeat length within the *FMR1* premutation group

	CGG repeat length	Vagal tone	Pragmatic Rating Scale score
CGG repeat length	1.00		
Vagal tone	0.37*	1.00	
Pragmatic Rating Scale score	-0.17	-0.37*	1.00

Partial correlations are presented, covarying for medication use

$*p < 0.05$

2.08], supporting vagal tone as a partial mediator underlying the relation between *FMR1* premutation genetic status and pragmatic ability. Unstandardized parameter estimates for the model are shown in Fig. 3.

### Associations with *FMR1* CGG Repeat Length

Finally, to investigate ties with *FMR1* molecular genetic indices, we conducted exploratory correlations between CGG repeat length, vagal tone, and pragmatic language scores in the *FMR1* premutation group<sup>1</sup>. Partial Pearson correlations were conducted, covarying for medication use. The length of the expanded CGG sequence was significantly associated with vagal tone ( $r = 0.37, p = 0.036$ ) but not pragmatic language skills ( $r = -0.17, p = 0.362$ ), see Table 3.

<sup>1</sup> *FMR1* CGG repeat length analyses were conducted on DNA isolated from either peripheral blood lymphocytes using standard methods (Qiagen, Valencia, CA) or whole blood dried blood spots (Adaye et al. 2014), using polymerase chain reaction and Southern Blot (Filipovic-Sadic et al. 2010; Tassone et al. 2008).

## Discussion

Investigations into the roots of pragmatic language impairment are timely. The autism epidemic has thrust pragmatic language impairment into the limelight, leading to increased awareness of the significant social, behavioral, and psychological consequences of pragmatic language impairment, and spurring the recent recognition of Social (Pragmatic) Communication Disorder as its own diagnostic category in DSM-5 (American Psychiatric Association 2013). This study investigated vagal tone, an index of parasympathetic “rest and digest” autonomic function, as a biophysiological pathway tied to pragmatic competence. We focused on the *FMRI* premutation as a genetic condition that may lend insight into mechanistic underpinnings of pragmatics that may generalize to other groups. Consistent with Polyvagal Theory, high vagal tone was associated with enhanced pragmatic competence in both women with the *FMRI* premutation and neurotypical women. Statistical mediation analysis indicated that the impact of *FMRI* premutation genetic status on pragmatic ability was carried through dampened vagal tone. This study opens new lines of inquiry into the physiological correlates of pragmatic ability that may be shared across the spectrum of pragmatic skills seen in both typical and atypical populations.

### Vagal Tone as a Biomarker for Pragmatic Competence

Given the wide variability in the clinical effects of the *FMRI* premutation, a major challenge to its clinical management is the lack of useful biomarkers that can lead to breakthroughs in the development of prevention/treatment efforts for this condition. Vagal tone is a promising potential biomarker because it is a quantitative, heritable trait that is established prenatally and shows within-individual stability across developmental periods (Calkins and Keane 2004; Doussard-Roosevelt et al. 2003; El-Sheikh 2005; Fracasso et al. 1994; Kleiger et al. 1991; Kupper et al. 2005). It can also be indexed reliably and non-invasively, making its application in clinical settings feasible. The present study provides preliminary support for vagal tone as a biomarker for pragmatic language deficits. We found, via statistical mediation analysis, that the impact of *FMRI* premutation carrier status on pragmatic language ability was carried through vagal tone. More work is needed for formal biomarker validation, which requires evidence of causal relationships between the putative biomarker and disease traits (Bonassi et al. 2001). We cannot infer causality from our cross-sectional design. Findings are nevertheless important, however, because they inform variability in the putative biomarker within population subgroups, contributing to the initial stages of biomarker validation.

Notably, prior research has failed to detect an association between dampened vagal tone and symptoms of anxiety and depression in women with the *FMRI* premutation (Klusek et al. 2017a). Thus, there appears to be a level of specificity, where only select features of the *FMRI* premutation phenotype are associated with dampened vagal tone. Findings suggest that the inclusion of vagal indices in future work may enhance understanding of *FMRI* gene-behavior associations by revealing indirect pathways that may mediate relationships.

A remaining question regards the specific *FMRI*-associated mechanisms driving the relationship between *FMRI* premutation group membership, vagal tone, and pragmatic ability. We lacked the sample size necessary to conduct formal mediation analyses testing putative pathways linking CGG repeat length, vagal tone, and pragmatics. However, our preliminary analyses did indicate an association between the size of the CGG expansion and vagal tone in the *FMRI* premutation group. Yet, CGG length was not correlated with pragmatic ability. Follow-up work in larger samples is needed to fully understand the nature of these relationships, as we may have been underpowered to detect small effects. Additionally, CGG length is only one indicator of *FMRI* gene function and other *FMRI* molecular genetic indices may be more closely linked with phenotypic variation. In particular, *FMRI* mRNA is of interest given its relationship with vagal tone detected in prior work (Klusek et al. 2017a).

Findings also support further investigation into the utility of vagal tone as a surrogate endpoint, or an “intermediate pathway” that may predict change in clinical endpoints. Despite major advances in understanding of the biology of *FMRI* conditions, the translation of molecular targets into therapeutics has been slowed by the lack of biomarkers that can sensitively index the impact of a new treatment on specific biological mechanisms that are correlated with clinical outcomes (see Jacquemont et al. 2014, for review). Vagal tone may be considered as a potential surrogate endpoint in future work because it is theoretically and empirically linked with core aspects of the phenotype and is associated with *FMRI* molecular genetic indices (Klusek et al. 2017a). There is also some evidence from the study of other clinical groups that vagal improvements may map onto positive behavior change, although findings are preliminary (Bagner et al. 2009; Graziano et al. 2012; Porges et al. 2013). The present study establishes an initial association between vagal tone and pragmatic language ability, suggesting that further validation work of vagal tone as a potential surrogate endpoint in *FMRI* conditions may be a fruitful direction.

It is notable that the detected association between vagal tone and pragmatic ability was not specific to the *FMRI* premutation; vagal tone accounted for pragmatic variability in the neurotypical controls as well. Thus, vagal tone could play a general mechanistic role in pragmatic language competence

that is relevant to both typical and atypical development. This generalizability across groups strengthens the potential utility of vagal tone as a biomarker for pragmatic language deficits. Modern conceptualizations of biomarkers recognize that biological underpinnings can cross diagnostic boundaries, which is consistent with frameworks such as the RDoC that emphasize dimensional-trait models that span the clinical and non-clinical spectrum (see Beauchaine and Thayer 2015 for discussion of transdiagnostic biomarkers). The detected association between vagal tone and pragmatics in control women (who did not carry premutation alleles on *FMRI*) does not preclude a role of *FMRI* in vagal regulation. Historically, *FMRI* has been studied almost exclusively within the context of fragile X-associated disorders, however, over the last decade it has become increasingly evident that *FMRI* is also highly important at the population level. Individuals in the general population, who do not carry abnormalities on *FMRI*, nevertheless show normal variability in the length of the CGG sequence, as well as in the expression of *FMRI* mRNA and *FMRI* protein (Chen et al. 2003; Ludwig et al. 2011; Pehrah 2012; Tassone et al. 2011; Wang et al. 2013). New evidence suggests that normal variation in these *FMRI* molecular genetic indices is associated with phenotypic variability in the general population, including associations with cognitive function and brain structure (Adamscheck et al. 2017; Mailick et al. 2014, 2017; Wang et al. 2013; Weghofer et al. 2012). Moreover, *FMRI* can effect widespread changes in background gene expression via its involvement in the translational regulation of over 800 other genes, which include one-third to one-half of the genes identified as ASD susceptibility genes (Darnell and Klann 2013; Darnell et al. 2011; Iossifov et al. 2012) as well as several genes implicated in autonomic regulation, such as *BDNF* and *CRHR1* (Allegrini et al. 2017; Gatt et al. 2009; Sumner et al. 2015; Yang et al. 2010). Thus, it is possible that *FMRI* gene function could relate to autonomic health in the general population and in other non-fragile X clinical groups, either through the direct effects of *FMRI* or via background gene interactions.

## Limitations and Directions

It is important to note that our cross-sectional design can only assume that the direction of influence was from vagal tone to pragmatic language. Our temporal assumption of variable ordering in the model is grounded in theory (i.e., Polyvagal Theory) and supported by empirical evidence that vagal tone is a heritable neurophysiological substrate that is established early in life (Calkins and Keane 2004; Doussard-Roosevelt et al. 2003; El-Sheikh 2005; Fracasso et al. 1994). Additionally, in fragile X syndrome, vagal dysfunction emerges in infancy and is therefore understood to represent a biological signature of *FMRI* gene dysfunction,

rather than a trait that emerges as a result of environmental factors (Roberts et al. 2012). However, our design prevents causal inferences and follow-up investigations are needed to confirm the presumed direction of effects.

There are some limitations related to the sample, such as lack of racial diversity, which may limit generalization of our findings. Also, our sample focused on women with the *FMRI* premutation who had a child with fragile X syndrome. While we did not detect any associations between parenting stress level and pragmatic skills or vagal tone, we are unable to completely parse out the stressors associated with parenting a child with a disability from the specific effects of the *FMRI* premutation. Future directions include investigation into the trajectory, developmental timing, and mechanisms of pragmatic language difficulties in early childhood, when intervention efforts may have the largest effects. Study strengths include the use of the Pragmatic Rating Scale, which provides a sensitive direct observation method for capturing pragmatic language ability across the normal and impaired range, as well as investigating mediation hypotheses of underlying mechanisms of pragmatic language.

## Conclusion

Focusing on the *FMRI* premutation as a genetic model for pragmatic language deficits, we found support for vagal tone as a biophysiological correlate of pragmatic ability across both women with the *FMRI* premutation as well as neurotypical controls. Statistical mediation analysis supported vagal tone as a mediator of the relationship between *FMRI* premutation genetic status and pragmatic language ability. Overall, findings suggest a link between healthy vagal function and social communication competence and point towards vagal tone as a biological factor that may relate to pragmatic problems in other clinical groups, such as ASD.

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**Author Contributions** JK conceived the study, led the data collection, analysis, interpretation, and drafted the manuscript. AJF provided consultation on the statistical models. JER provided guidance on the study design, data collection, and interpretation. All authors contributed to the interpretation of results and critical revising of the manuscript. All authors read and approved the final manuscript.

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## Compliance with Ethical Standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical Approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and with the 1964 Helsinki declaration and its later amendments.

**Informed Consent** Informed consent was obtained from all individual participants included in the study.

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