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Atypical Vocal Quality in Women with the *FMR1* Premutation: An Indicator of Impaired Sensorimotor Control

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Abstract

Women with the *FMR1* premutation are susceptible to motor involvement related to atypical cerebellar function, including risk for developing fragile X tremor ataxia syndrome. Vocal quality analyses are sensitive to subtle differences in motor skills but have not yet been applied to the *FMR1* premutation. This study examined whether women with the *FMR1* premutation demonstrate differences in vocal quality, and whether such differences relate to *FMR1* genetic, executive, motor, or health features of the *FMR1* premutation. Participants included 35 women with the *FMR1* premutation and 45 age-matched women without the *FMR1* premutation who served as a comparison group. Three sustained /a/ vowels were analyzed for pitch (mean F0), variability of pitch (standard deviation of F0), and overall vocal quality (jitter, shimmer, and harmonics-to-noise ratio). Executive, motor, and health indices were obtained from direct and self-report measures and genetic samples were analyzed for *FMR1* CGG repeat length and activation ratio. Women with the *FMR1* premutation had a lower pitch, larger pitch variability, and poorer vocal quality than the comparison group. Working memory was related to harmonics-to-noise ratio and shimmer in women with the *FMR1* premutation. Vocal quality abnormalities differentiated women with the *FMR1* premutation from the comparison group and were evident even in the absence of other clinically evident motor deficits. This study supports vocal quality analyses as a tool that may prove useful in the detection of early signs of motor involvement in this population.

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Keywords

voice; neurodegenerative disease; fundamental frequency; acoustic analysis; fragile X-associated tremor/ataxia syndrome

The *fragile X messenger ribonucleoprotein 1* (*FMR1)* gene is responsible for the production of FMRP, which is a protein that regulates the translation of neuronal proteins vital to synaptic function (Hagerman & Hagerman, 2013). The number of cytosine-guanine-guanine (CGG) trinucleotide repeats in *FMR1* varies across individuals, with the typical range being 5–44 repeats (Darnell et al., 2011; Maddalena et al., 2001; Spector et al., 2021). Expansion of the CGG sequence to 55–200 repeats is known as the *FMR1* premutation. Expanded CGG sequence and associated elevated levels of *FMR1* mRNA and abnormal RAN translation products result in various biological dysfunctions that can manifest as distinct cognitive and physiological phenotypes ranging across the lifespan (Hessl et al., 2005; Hocking et al., 2017). The *FMR1* premutation is highly prevalent, occurring in 1 in 113–178 women (Hantash et al., 2011; Seltzer et al., 2012; Toledano-Alhadef et al., 2001). Though women with the *FMR1* premutation were previously thought to be clinically unaffected, it is now evident that the genotype is associated with a variety of age-related health concerns, including early menopause and motor deficits; additionally, anxiety, depression, and executive function deficits are variably present and increase with age (Cordeiro, Abucayan, Hagerman, Tassone, & Hessl, 2015; Hagerman et al., 2018; Klusek, Hong, Sterling, Berry-Kravis, & Mailick, 2020; Maltman et al., 2022; Moser, Schmitt, Schmidt, Fairchild, & Klusek, 2021; O'Keefe et al., 2015; O'Keeffe et al., 2019; Roberts et al., 2016; Wittenberger et al., 2007). Furthermore, women with the *FMR1* premutation are at risk for neurodegenerative disorders, including fragile X-associated tremor/ataxia syndrome (FXTAS), which affects 8–16% of women with the *FMR1* premutation over the age of 50 (Coffey et al., 2008; Rodriguez-Revenga et al., 2009). FXTAS is associated with atrophy and white matter lesions in the cerebrum and cerebellum, and the clinical profile is characterized by intention tremors, balance problems, muscle stiffness, slow movement, and neurocognitive difficulties (Greco et al., 2006; Leehey, 2009).

Women with the *FMR1* premutation may experience an atypical motor profile regardless of FXTAS expression (Kraan et al., 2013; O'Keefe et al., 2015). Emerging studies on motor control in middle-aged women with the *FMR1* premutation, even those without FXTAS, have demonstrated deficits in various domains, including postural control, oculomotor inhibition, and precision sensorimotor control (Kraan et al., 2013, 2014b; McKinney et al., 2020, 2019; Moser et al., 2021; O'Keefe et al., 2015; O'Keeffe et al., 2019; Park et al., 2019; Shelton et al., 2014; Wang et al., 2021; Wang, Khemani, Schmitt, Lui, & Mosconi, 2019). The presence of these motor deficits has been hypothesized to be a marker of early or atypical aging as well as a precursor to the development of FXTAS, though this is not yet clear. The risk for developing FXTAS increases with higher CGG repeat lengths (Greco et al., 2006; Tassone et al., 2007), and several studies have reported that motor impairments are associated with higher CGG repeat lengths (Kraan et al., 2013; McKinney et al., 2019; O'Keeffe et al., 2019; Wang et al., 2019). Additionally, activation ratio (the percentage of

cells with the normal allele on the active X chromosome) has been found to be associated with motor control in women with the *FMR1* premutation (O'Keefe et al., 2015).

The methods to identify motor impairments are often complex, requiring expensive equipment (i.e., force platforms, precision load cells) and analyses that currently present a barrier to feasible wide-scale application to the identification of early disease or aging across a large range of people. The development of methods that can identify early, subclinical signs of motor difficulties and that are accessible to medical professionals as well as easy to administer is key to the early detection and enhanced clinical management of FXTAS and/or general age-related motor deficits associated with the *FMR1* premutation.

One established, sensitive, early motor marker for neurodegenerative movement disorders that has not yet been applied to the *FMR1* premutation is vocal quality analysis. The complexity of the phonatory system and the motor sensitivity required for producing the vast range of sounds that make up human speech makes phonation an ideal system to examine subtle motoric changes that may precede clinical deficits (Fagherazzi, Fischer, Ismael, & Despotovic, 2021; Toth et al., 2017; Tracy, Özkanca, Atkins, & Hosseini Ghomi, 2020). Therefore, subtle yet quantifiable changes in vocal quality could theoretically be used to identify the early stages of disease or risk for later disease onset, thus presenting an opportunity for early diagnosis, intervention, and prevention. Vocal quality parameters are highly sensitive to structural and functional changes from both normative aging and presence of pathologies (Harel, Cannizzaro, & Snyder, 2004; Hlavnika et al., 2017; Midi et al., 2008; Rusz et al., 2011). Age-related changes can be detected using vocal quality analyses, and occur as early as the age of 50 (Russell, Penny, & Pemberton, 1995; Stathopoulos, Huber, & Sussman, 2011), though a recent meta-analysis suggested that marked changes happen after 80 years of age (Rojas, Kefalianos, & Vogel, 2020). Consequently, vocal quality abnormalities may be the first observable manifestation of neurodegenerative disorders (Rahn, Chou, Jiang, & Zhang, 2007), and are often detectable prior to deficits on direct kinematic motor measures (Fagherazzi et al., 2021; Toth et al., 2017; Tracy et al., 2020). A major advantage of vocal quality analyses is that they provide a feasible, low-cost method for early detection of sensorimotor abnormalities, as special equipment is not required (e.g., vocal samples can be reliably assessed from smartphone recordings; Grillo, Brosious, Sorrell, & Anand, 2016; Uloza et al., 2015), and free, user-friendly software is available (e.g., Praat; Boersma & Weenink, 2018). Interpretation for clinicians who are not experts in voice disorders is simplified by the availability of published norms (Goy, Fernandes, Pichora-Fuller, & Van Lieshout, 2013).

Several vocal features can be indexed from a simple sustained phonation task, such as production of the vowel /a/ for several seconds. These features include fundamental frequency (i.e., F0, pitch) and standard deviation of F0, harmonics-to-noise ratio (the ratio between periodic and non-periodic speech), jitter (perturbation related to F0), and shimmer (perturbation related to vocal intensity). Several studies have found that these features successfully distinguish those with neurodegenerative disorders, including dementia-related diseases and motor diseases such as Parkinson's, from controls (Burk & Watts, 2019; Jiménez-Jiménez et al., 1997; López-de-Ipiña et al., 2013; Martínez-Nicolás, Llorente, Martínez-Sánchez, & Meilán, 2021; Meilán et al., 2014; Midi et al., 2008; Ramig, Titze,

Scherer, & Ringel, 1988; Sauder, Bretl, & Eadie, 2017; Tracy et al., 2020; Tsanas, Little, McSharry, Spielman, & Ramig, 2012). For example, using vocal quality analyses, Meilán et al. (2014) accurately distinguished participants with Alzheimer's disease from controls with 85% accuracy. In another report, Tsanas et al. (2012) used vocal quality analyses to distinguish participants with Parkinson's disease from controls with 99% accuracy (Meilán et al., 2014; Tsanas et al., 2012). These findings suggest that the analysis of vocal quality abnormalities can be used to reliably discriminate between healthy individuals and those with various forms of neurodegenerative disease.

Present Study

Vocal quality analyses can differentiate and predict a variety of neurodegenerative conditions, can easily be extracted and quantified from a short vowel production sample, and are non-invasive and inexpensive to collect. Therefore, it stands to reason that the application of vocal quality analysis in women with the *FMR1* premutation may lend insight into subtle deficits in motor control that may not be detectable with tasks involving other motor systems. The present study addressed four research questions:

1. *Does the vocal quality of women with the FMR1 premutation differ from control women?* Based on prior evidence of motor dysfunction in women with the *FMR1* premutation, we predicted that aspects of vocal quality, which index sensorimotor control of the phonatory system, would be affected in this population.

2. *Is vocal quality associated with age within women with the FMR1 premutation or control women?* Given prior evidence of age-specific associations suggesting potentially accelerated aging in the *FMR1* premutation (e.g., Moser et al., 2021; Sterling, Mailick, Greenberg, Warren, & Brady, 2013), we predicted that women with the *FMR1* premutation would show age-related decline in vocal quality. We did not predict similar age associations in controls, given that our average sample age was younger than when age-related changes in vocal quality typically emerge.

3. *Is vocal quality associated with motor function, physical health, and executive function?* We posed this question to better understand the interface between vocal quality dysfunction and other key features of the *FMR1* premutation phenotype that may be linked to the later development of FXTAS. We predicted that vocal quality dysfunction (i.e., lower mean F0, larger standard deviation of F0, lower harmonics-to-noise ratio, increased jitter and shimmer) would be linked with deficits in motor function and physical health, as indicated by direct quantitative assessment of balance and self-reported measures of functional tremor symptoms and physical health limitations. We also predicted that vocal quality dysfunction would be associated with poorer performance on executive measures of working memory, inhibition, and attention (O'Keefe et al., 2015; Storey et al., 2021).

4. *Is vocal quality associated with FMR1-related molecular genetic indices?* Based on prior evidence suggesting associations between molecular genetic indices

and motor dysfunction, we hypothesized that increased CGG repeat length and higher activation ratio would be associated with increased vocal quality dysfunction.

Methods

Participants

Participants were 35 women with the *FMR1* premutation and a comparison group of 45 women who did not carry the *FMR1* premutation. Groups were closely matched on age ($t[78] = 0.09$, $p = .928$, $d = -0.02$), with a mean age of 48.12 years (range = 26–73). All women were enrolled in a larger study focused on language phenotypes in women with the *FMR1* premutation. Women with the *FMR1* premutation had 55–200 CGG repeats on the 5' untranslated region of *FMR1*, as confirmed through genetic testing, and had children with fragile X syndrome, the *FMR1* premutation, or a family history of fragile X-associated conditions. Although those with FXTAS were not explicitly excluded from the study, none of the women with the *FMR1* premutation had a clinical FXTAS diagnosis, according to self-report. Women in the comparison group did not have a family history of fragile X-associated conditions and completed genetic testing through the larger study to rule out the *FMR1* premutation. The comparison group was comprised of either mothers of children with autism spectrum disorder or mothers of children without any diagnosed developmental disabilities. In the present study, these groups were collapsed given that both groups represented women without *FMR1*-related conditions and initial analyses indicated that these groups did not differ on any of the vocal quality parameters examined. The majority of the sample identified as White (89%), with no differences in the racial distribution across the groups ($\chi^2(1, N=79)=2.14$, $p = .143$). Women with the *FMR1* premutation were recruited through national organizations, social media, word of mouth, or prior study participation (Klusek, Fairchild, & Roberts, 2019). Women in the comparison group were recruited through flyers in pediatricians' offices, social media, or word of mouth.

Procedure

Participants traveled to the University of South Carolina to complete testing in the university laboratory and provided written consent prior to study participation. Study protocol was approved by the university's Institutional Review Board. Questionnaire data were gathered in the two weeks preceding the assessment via a REDCap survey (Harris et al., 2019, 2009) and included questions on demographics and current medication use. The vocal quality sample was administered approximately one hour into the assessment, following administration of standardized cognitive and language measures. Buccal swabs for genetic testing were collected at the end of the assessment.

Measures

Vocal Quality Indices—Vocal quality was indexed from sustained vowel samples and consisted of values averaged from three repetitions of the vowel /a/, sustained for 5 seconds. All voice samples were collected with a HOTEC H-W07 professional microphone positioned at a 45-degree angle, approximately eight inches from the participant's mouth. Audio files were analyzed in Praat using system default settings (Boersma & Weenink,

2018). Sustained vowels were trimmed to segment and isolate the medial three seconds of the vowel. System default settings were used to calculate values for mean F0 (pitch) and standard deviation of F0 (pitch variability), harmonics-to-noise ratio (overall vocal quality), jitter % (perturbation related to frequency), and shimmer % (perturbation related to intensity).

Indices of Motor Function and Physical Health

Functional Tremor Disability Questionnaire.: The Functional Tremor Disability Questionnaire assesses tremor symptoms related to limitations of daily function (Louis et al., 2000) and has previously been used to assess tremor symptoms in women with the *FMR1* premutation (Jacquemont et al., 2004; Klusek et al., 2022, 2017). Participants are asked to rate the amount of difficulty they experience completing various everyday tasks (e.g., tying shoes, threading a needle) on a 3-point scale, ranging from “no problem” to “I need to modify the way I perform this task; the task is difficult.” A higher overall score reflects more severe functional tremor symptoms and increased limitations. This questionnaire has good concurrent validity and test-retest reliability, and it is associated with direct assessments of tremor (Louis et al., 2000).

NIH Toolbox Standing Balance Test.: The balance scale of the National Institute of Health (NIH) Motor Toolbox (Reuben et al., 2013) is a measure of balance as indicated by postural sway while participants hold five progressively demanding poses while wearing an accelerometer at waist level (Rine et al., 2013). Raw scores were converted to *T*-scores, which corrected for age and other demographic variables. Lower scores indicate poorer balance (Gershon et al., 2013). This task has good test-retest reliability and acceptable criterion validity (Peller et al., 2022). Similar balance tasks have been used to assess motor deficits in women with the *FMR1* premutation (Kraan et al., 2013; O’Keefe et al., 2015).

RAND-36 Health Short Form Survey.: The RAND 36-Item Health Short Form Survey assesses eight health components measuring quality of life related to physical and mental health (Hays, Sherbourne, & Mazel, 1993), and has previously been used to characterize health in women with the *FMR1* premutation (Mailick et al., 2018). A physical health component summary score (RAND Health PCS) was computed as described by Ware et al. (1994). Standardized scores for the general health, physical functioning, bodily pain, and role limitations due to physical health problems are positively weighted, and the remaining subscales are negatively weighted (role limitations due to emotional health problems, emotional well-being, social functioning, and energy and fatigue); the weighted scores are then summed to produce a component summary score that reflects physical aspects of health (Ware, Kosinski, & Keller, 1994). Statistically, this score avoids floor and ceiling effects relative to the individual subscale scores. A lower score indicates more issues with physical health. This measure has high internal consistency and high convergent validity (VanderZee, Sanderman, Keyink, & de Haes, 1996).

Executive Function Indices

Spatial Addition.: Working memory was measured with the spatial addition subtest of the Wechsler Memory Scale – Fourth edition (WMS-IV; 48). This is a visual addition task in

which the participant looks at two subsequent grids with blue and red circles and is then asked to add or subtract the location of the circles based on a set of rules. The spatial addition task measures visual-spatial working memory. Standard scores were computed based on a normative sample. Two women with the *FMR1* premutation were older than the normative sample for this subtest; therefore, standard scores were not calculated for these participants, and they were not included in analyses with the spatial addition scores. This subtest has good ecological validity (Drozdick & Cullum, 2011) as well as high internal consistency, ranging from 0.89 to 0.93 (Holdnack, Drozdick, & Wechsler, 2009).

Hayling Sentence Completion Test.: Inhibition was measured using the Hayling Sentence Completion Test converted error score (Burgess & Shallice, 1997). In the first part of this task, the examiner reads a series of 15 sentences, each of which has the last word missing. The participant provides a word that completes the sentence as quickly as possible. In the second part, the examiner reads a different series of 15 sentences with the last word missing, but the participant provides a word that is unconnected to each sentence as quickly as possible, which requires inhibition of prepotent responses. Responses from the second set are scored for category A errors (responses that are connected to the sentence) and category B errors (responses that are somewhat connected). The total number of category A and B errors are each converted using a scale provided on the protocol; these converted scores are summed, yielding a converted A+B error score. Error scores can range from 0 to 78, with a higher score reflecting impaired inhibition. This measure has good construct validity and adequate test-retest reliability (Andrés & Van der Linden, 2000; Burgess & Shallice, 1997).

Brown Attention-Deficit Disorder Scales.: Attention was assessed with the Brown Attention-Deficit Disorder (ADD) Scales, Ready Score-Adult (T. E. Brown, 1996). This 40-item scale assesses a range of inattention symptoms. Scores can range from 0 to 120, with higher scores reflecting more symptoms of inattention. This scale demonstrates high internal consistency (.96) and evidence of validity (T. E. Brown, 2001).

FMR1 Molecular Genetic Variables—DNA was isolated from buccal samples using standard methods. CGG repeat length was determined by polymerase chain reaction (PCR) using the Asuragen AmplideX® Kit (Chen et al., 2010; Grasso et al., 2014). Activation ratio was determined using Asuragen AmplideX® FMR1 mPCR Kit (Chen et al., 2011). Analyses were conducted in the laboratory of Dr. Berry-Kravis at Rush University Medical Center. Due to insufficient amounts of DNA for analyses, one participant was missing CGG and activation ratio data and two participants were missing activation ratio data.

Medication Use—Participants completed an in-house questionnaire that inquired about current medication use that was used to quantify the use of medications known to affect vocal quality (i.e., antihistamines, hormones, corticosteroids, antivirals, and tricyclic antidepressants; Abaza, Levy, Hawkshaw, & Sataloff, 2007; Murry, McRoy, & Parhizkar, 2007)

Data Analysis

All data analyses were conducted in R (R Core Team, 2020). Our first research question regarding group differences in voice quality variables was analyzed using linear regression models. Prior to analyses, all variables were examined for normality and descriptive statistics were computed (Table 2). All sustained vowel variables were normally distributed, except the standard deviation of F0 and jitter, which were both positively skewed. These variables were therefore analyzed using a generalized linear model with a gamma distribution and a log-link function which was determined to be an appropriate fit for the skewed distribution. Although the groups did not differ on the percent using medications that can affect vocal quality, $\chi^2[1, N=80]=0.00$, $p = .977$, we further controlled for this potential confound by controlling for medication use in analyses, as reflected by a dichotomous variable (present/absent). The Benjamini-Hochberg false discovery rate correction was applied at the level of the model F to account for multiple comparisons (Benjamini & Hochberg, 1995). Partial eta-squared was calculated as a measure of effect size and interpreted as 0.01=small effect, 0.06=medium effect, 0.14=large effect.

We addressed the second research question regarding the relationship between vocal quality and age within women with the *FMR1* premutation and control women using general linear models for all variables, except models with standard deviation of F0 and jitter as outcome variables, which were examined with a generalized linear model with a gamma distribution and a log-link function. All models assessing vocal quality and age controlled for medication use. The third research question addressing associations between vocal quality and indices of motor and executive function within the *FMR1* premutation group and control groups was also addressed using linear models or generalized linear models controlling for medication use. Specifically, models with the standard deviation of F0 and jitter as outcome variables were analyzed with generalized linear models with a gamma distribution and a log-link function to account for non-normality. Because this aim is exploratory in nature, we did not apply a correction to the p-values.

Finally, we explored associations between CGG repeats, activation ratio, and vocal quality parameters in women with the *FMR1* premutation using linear regression or generalized linear models. CGG repeats and activation ratio were analyzed in separate models. Medication use was controlled for in all models. For the models predicting standard deviation of F0 and jitter, we applied a gamma distribution with a log-link function which best fit the models. Given prior findings on curvilinear associations between CGG repeats and behavioral aspects of the *FMR1* premutation phenotype (Klusek et al., 2018; Mailick, Hong, Greenberg, Smith, & Sherman, 2014), we probed higher order polynomial CGG terms; no higher order values were significant, so they were not retained in the models.

Results

Group Differences in Vocal Quality

Women with the *FMR1* premutation had a significantly lower mean F0 with a medium effect size ($F[1,77]=9.32$, FDR-corrected $p = .015$, $\eta_p^2 = .11$), indicating a lower pitch. Higher standard deviation of F0 was also observed with a medium effect size ($F[1,77]=5.15$,

FDR-corrected p =.026, η_p^2 = .07), indicating poorer vocal control during the sustained vowel. Finally, women with the *FMR1* premutation had a lower harmonics-to-noise ratio (F[1,77]=6.89, FDR-corrected p =.043, η_p^2= .08) than the comparison group with a medium effect size, indicating poorer vocal quality. Groups did not differ in jitter (F[1,77]=1.20, FDR-corrected p =.339, η_p^2 = .04) or shimmer (F[1,77]=0.93, FDR-corrected p =.339, η_p^2 = .01). Group differences are depicted in Figure 1.

Relationship between Vocal Quality and Age

Within women with the *FMR1* premutation, age was not associated with mean F0 (F[2,32]=0.55, p = .473, η_p^2 = .02), standard deviation of F0 (F[2,32]=1.32, p = .258, η_p^2 = .04), harmonics-to-noise ratio (F[2,32]=0.35, p = .559, η_p^2 = .01), jitter (F[2,32]=0.45, p = .506, η_p^2 = .05), or shimmer (F[2,32]=1.57, p = .219, η_p^2 = .05).

Within control women, age was not associated with mean F0 (F[2,42]=1.61, p = .287, η_p^2 = .03), standard deviation of F0 (F[2,42]=2.59, p = .115, η_p^2 = .07), harmonics-to-noise ratio (F[2,42]=0.05, p = .823, η_p^2 = .00), jitter (F[2,42]=0.27, p = .608, η_p^2 = .02), or shimmer (F[2,42]=1.67, p = .204, η_p^2 = .04).

Relationship between Vocal Quality and Motor Function, Physical Health, and Executive Function

Of the executive function variables, lower scores on the spatial addition task (i.e., working memory) was associated with poorer vocal quality, specifically harmonics-to-noise ratio (p = .003, η_p^2 = .30) and shimmer (p = .003, η_p^2 = .29) within women with the *FMR1* premutation; see Figure 2. No significant associations were observed with the motor and physical health variables for this group (see Table 2 for coefficients for women with the *FMR1* premutation). Among the control women, lower scores on the Brown ADD questionnaire (i.e., attention) was associated with lower mean F0 (p = .045, η_p^2 = .10), and lower NIH Balance scores were associated with increased standard deviation of F0 (p = .020, η_p^2 = .14; see Table 2 for coefficients for control women).

Association between Vocal Quality and *FMR1* Indices

There were no significant associations between the molecular genetic indices and vocal quality. Within women with the *FMR1* premutation, CGG repeat length was not associated with mean F0 (F[2,31]=0.11, p = .741, η_p^2 = .00), standard deviation of F0 (F[2,31]=2.55, p = .121, η_p^2 = .06), harmonics-to-noise ratio (F[2,31]=1.07, p = .310, η_p^2 = .03), jitter (F[2,31]=2.09, p = .159, η_p^2 = .14), or shimmer (F[2,31]=3.03, p = .092, η_p^2 = .09). Activation ratio also was not associated with mean F0 (F[2,29]=0.04, p = .848, η_p^2 = .00), standard deviation of F0 (F[2,29]=3.99, p = .055, η_p^2 = .10), harmonics-to-noise ratio (F[2,29]=0.52, p = .478, η_p^2 = .02), jitter (F[2,29]=0.23, p = .636, η_p^2 = .02) or shimmer (F[2,29]=0.01, p = .937, η_p^2 = .00). Following (Leehey et al., 2008), we also probed for CGG effects while controlling for activation ratio, and inferences did not change for any model.

Discussion

Emerging research suggests that women with the *FMR1* premutation, even those without a diagnosis of FXTAS, have an atypical motor profile as demonstrated by a variety of subtle and often subclinical symptoms (Kraan et al., 2013, 2014b; McKinney et al., 2020, 2019; Moser et al., 2021; O'Keefe et al., 2015; O'Keeffe et al., 2019; Park et al., 2019; Shelton et al., 2014; Wang et al., 2021, 2019). The present study took a novel approach toward characterizing these motor deficits via vocal quality analyses, which are sensitive to differences between individuals with and without neurodegenerative diseases as well as subtle changes in aging (Harel et al., 2004; Hlavnika et al., 2017; Midi et al., 2008; Rusz et al., 2011). We found that women with the *FMR1* premutation differed from a comparison group comprised of women who did not carry *FMR1* mutations on measures of pitch, pitch stability, and overall vocal quality. We also found that, among women with the *FMR1* premutation, overall vocal quality was related to working memory. These findings contribute to the burgeoning body of literature on motor issues within women with the *FMR1* premutation and have implications for the utility of vocal quality analyses within this population.

Consistent with our hypotheses, we found that women with the *FMR1* premutation differed from the comparison group on several vocal quality parameters. Specifically, women with the *FMR1* premutation had a lower mean F0, larger standard deviation of F0, and lower harmonics-to-noise ratio, indicating atypical pitch, heightened pitch variability, and overall poorer voice quality. This finding adds to growing evidence that women with the *FMR1* premutation who do not have FXTAS experience motor problems (Kraan et al., 2013, 2014b; McKinney et al., 2020, 2019; Moser et al., 2021; O'Keefe et al., 2015; O'Keeffe et al., 2019; Park et al., 2019; Shelton et al., 2014; Wang et al., 2021, 2019) by demonstrating that vocal quality – reflecting sensorimotor control of the phonatory system – is also affected in this group. The vocal quality differences observed in this study may arise from neural structures that support speech motor control and phonation, such as the cerebellum (Song et al., 2022), which has been suggested to be implicated in women with the *FMR1* premutation, even those without FXTAS (Kraan et al., 2013; Storey et al., 2021). Notably, vocal quality dysfunction was evident in the present sample of women with the *FMR1* premutation prior to the emergence of other clinically evident motor problems; the present sample did not have clinical diagnoses of FXTAS and did not have balance, functional tremor, or physical health scores indicative of clinical motor problems. This finding is consistent with prior studies suggesting that vocal quality analyses are sensitive to early signs of disease relative to direct kinematic measures (Fagherazzi et al., 2021; Toth et al., 2017; Tracy et al., 2020), and therefore may be useful in the early detection of emergent motor problems.

Poorer working memory skills were associated with several vocal quality features within women with the *FMR1* premutation, including lower harmonics-to-noise ratio and higher shimmer (perceived as hoarseness). Within those with the *FMR1* premutation, deficits in executive function skills, such as working memory, are an early indicator of those who later develop FXTAS (Famula et al., 2022; Kogan & Cornish, 2010), and working memory deficits in women with the *FMR1* premutation are associated with motor impairments such as gait variability and tremor (Kraan et al., 2014a; Storey et al., 2021). Thus, this finding

suggests that vocal quality deficits in women with the *FMR1* premutation may align with other established early features of FXTAS (i.e., executive dysfunction), supporting vocal quality analysis as a potentially useful indicator of risk for the later development of FXTAS. Moreover, we did not find similar associations between working memory and vocal quality in control women, which provides a preliminary indication of group-specific associations. A remaining question for future research is whether vocal quality and working memory difficulties originate from shared neurocognitive mechanisms within women with the *FMR1* premutation. Specifically, the sensorimotor cortex is implicated in voice production, and sensorimotor processes are theorized to play a role in visuospatial working memory (Olthoff, Baudewig, Kruse, & Dechent, 2008; Wilson, 2001). Thus, examining sensorimotor cortex changes among women with the *FMR1* premutation may be a fruitful avenue for future research.

While our finding of atypical vocal quality in women with the *FMR1* premutation is in itself indicative of motor dysfunction, we did not observe concurrent associations between vocal quality indices and other more frank motor problems (i.e., balance deficits and tremor). By focusing on women with the *FMR1* premutation who did not have FXTAS or frank motor involvement in the present study, we were able to add to an emerging evidence base supporting the presence of sensorimotor control deficits (indicated in the present study by vocal quality abnormalities) in women with the *FMR1* premutation who do not have FXTAS. However, it remains unclear whether the atypical vocal quality detected in the present study reflects a preclinical marker for the later development of FXTAS or a broader premutation-associated motor phenotype. As vocal quality is a predictor of neurodegenerative disorders (Harel et al., 2004; Hlavnika et al., 2017; Midi et al., 2008; Rahn et al., 2007; Rusz et al., 2011), atypical vocal quality may be explored in future studies as a potential precursor to FXTAS. Specifically future focus on an older sample or a sample symptomatic for FXTAS may better clarify how vocal quality dysfunction maps onto other motor symptoms seen in the *FMR1* premutation; the low level of tremor and balance problems in our sample may have prevented the detection of such an association. Vocal changes in FXTAS have been described in case studies, which note perceived vocal tremor (Fay-Karmon & Hassin-Baer, 2019). Future studies should compare vocal quality to other measures that differentiate women with the *FMR1* premutation from controls (e.g., measures of sensorimotor precision control or postural control; 23,24) as well as examine the predictive value of vocal quality on other aspects of motor control longitudinally, both within and outside of the context of FXTAS.

Vocal quality indices were not related to age in women with the *FMR1* premutation or control women. This may be because our participants, on average, were overall younger than the typical age of onset for vocal quality changes (Russell et al., 1995; Stathopoulos et al., 2011). It may also be the case that the atypical vocal quality observed in women with the *FMR1* premutation is a marker for neuropathological motor issues that are not age-linked. Further research is needed to clarify effects, such as longitudinal research and focus on older samples.

Finally, we did not detect significant associations between vocal quality and CGG repeat length or activation ratio within women with the *FMR1* premutation. Previous studies

have shown that CGG repeat length, without or without controlling for activation ratio, is associated with ataxia and postural control in women with the *FMR1* premutation (Kraan et al., 2013; Leehey et al., 2008), and activation ratio has previously been linked to balance in women with and without FXTAS (O'Keefe et al., 2015). Our sample size was relatively small and we did not have participants who had higher premutation CGG repeat lengths which occur less frequently in the population; this limited range may have affected our ability to detect associations with CGG repeats. Future research should aim to elucidate potential associations between vocal quality and *FMR1* indices in a larger sample size, as characterization of motor features and their associations with molecular indices is critical to defining the expression of the *FMR1* premutation phenotype.

Strengths, Limitations, and Future Directions

This study presents several strengths. Our use of vocal quality analyses for a sustained vowel task is a key strength of this study, as it represents a quick and inexpensive method for identifying vocal quality impairments. Recent work has highlighted the development of automatic vocal quality analyses that use machine learning algorithms to yield computer-aided diagnoses of various diseases, including Parkinson's disease (Gómez-García, Moro-Velázquez, & Godino-Llorente, 2019; Madruga, Campos-Roca, & Pérez, 2021; Vashkevich & Rushkevich, 2021). A key future direction for this research includes exploring the utility of these automated vocal quality analyses for women with the *FMR1* premutation. Another strength was our inclusion of both direct and self-report measures of motor and executive function indices, which provided a muti-modal approach toward understanding the association between these constructs and vocal quality. We employed measures of functional tremor and physical health that capture functional deficits experienced in daily life and therefore have strong clinical relevance; however, the use of direct assessment measures of tremor or physical health in future work may illuminate more nuanced relationships with vocal quality. Direct assessment of FXTAS, instead of reliance on self-reported clinical diagnoses, may have been useful in allowing us to further characterize our sample. Future research should include FXTAS measures, as well as examine vocal quality among those who develop FXTAS, which would enhance our understanding of the utility of phonatory analyses as an early marker of FXTAS. Relatedly, without longitudinal data, the emergence and trajectory of atypical vocal quality remain unclear. This study also does not address neural underpinnings; future studies might explore atypical aging via cerebellar changes as a potential mechanism for the vocal quality differences observed in the present study, given that cerebellar dysfunction is implicated in aging among those with the *FMR1* premutation without FXTAS (S. S. G. Brown, Basu, Whalley, Kind, & Stanfield, 2018; Kraan et al., 2013; Storey et al., 2021). Additionally, the present study was designed to document group differences in vocal quality and begin to elucidate the relationships between vocal abnormalities in the *FMR1* premutation and other aspects of the phenotype, and therefore lacked the sample size to test associations within both groups within the same statistical model. We also did not correct for multiple models, as these analyses were exploratory. Finally, our sample lacked racial diversity, which limits the generalizability and should be addressed in future work.

Conclusions

This study documents vocal quality abnormalities in women with the *FMR1* premutation which are detectable prior to the onset of other clinically evident motor problems. Future studies may contribute to our understanding of the utility of vocal quality analysis as a potential preclinical marker for neurodegeneration in women with the *FMR1* premutation. Early detection of the onset of motor involvement in women with the *FMR1* premutation may aid in the early initiation of prevention or treatments to promote age-related health in this population.

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Data Availability Statement:

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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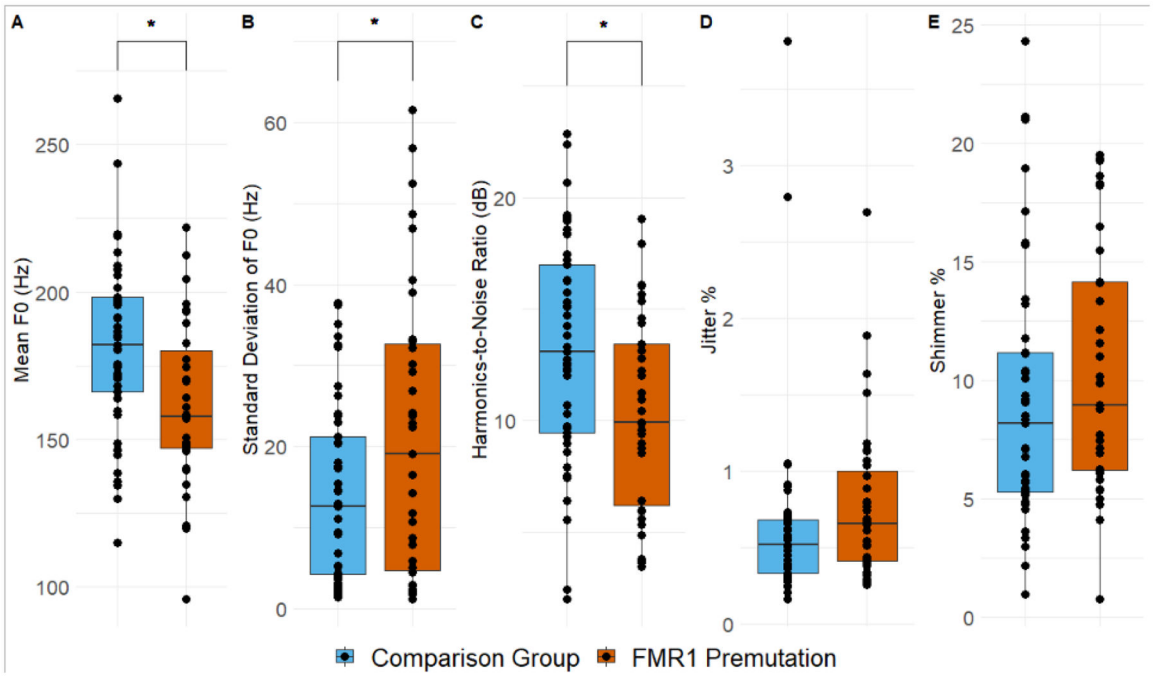

Figure 1.

Group Differences in Mean F0 (A), Standard Deviation of F0 (B), Harmonics-to-Noise Ratio (C). Jitter % (D), and Shimmer % (E)

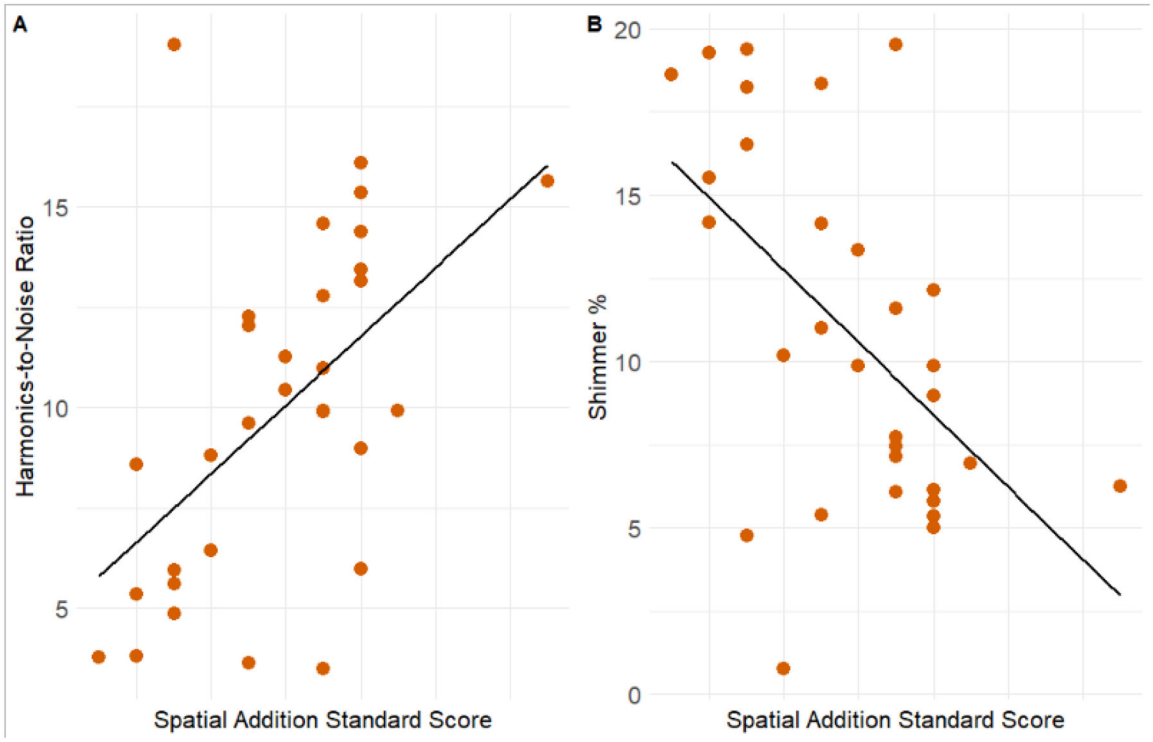

Figure 2.

Association Between Poorer Working Memory and Lower Harmonics-to-Noise Ratio (A) and Increased Vocal Intensity Perturbation (B) in Women with the *FMR1* Premutation

Table 1

Descriptive Statistics

	Group	
	***FMR1* Premutation** ***n*=35** ***M* (*SD*) [range]**	**Comparison Group** ***n*=45** ***M* (*SD*) [range]**
Vocal Quality Indices		
Mean F0 (Hz)	161.99 (27.56), 96.02–221.86	182.29 (30.91), 115.05–265.66
Standard deviation of F0 (Hz)	21.28 (17.88), 1.20–61.55	14.20 (11.60), 1.41–37.73
Harmonics-to-noise ratio (dB)	10.37 (4.36), 3.48–19.04	13.16 (4.95), 2.02–22.85
Jitter %	0.78 (0.52), 0.26–2.69	0.65 (0.63), 0.16–3.81
Shimmer %	10.37 (5.21),0.77–19.51	9.22 (5.55), 0.96–24.33
Motor and Executive Function Indices		
Tremor Disability Questionnaire Score	1.07 (1.57), 0–6	1.33 (2.42), 0–9
RAND Health PCS	51.11 (7.72), 34.08–61.69	50.38 (9.89), 25.17–62.63
NIH Balance Standard Score	97.35 (15.56), 79–139	102.20 (17.41), 59–144
WMS-IV Spatial Addition Standard Score	99.66 (13.22), 75–135	99.76 (15.49), 65–125
Hayling Converted Error Score	4.45 (5.78), 0–32	4.34 (5.33), 0–25
Brown ADD Total Score	34.97 (21.01), 4–74	27.12 (22.31), 3–106
FMR1 Genetic Indices		
CGG repeats	91.85 (13.31), 64–117	32.34 (4.30), 25–43
Activation ratio	0.46 (0.16), 0.05–0.75	N/A
Percentage using of medication(s) that can affect vocal quality[1]	57%	60%

Note.

[1]Drug classes that affect vocal quality include antihistamines, hormones, corticosteroids, antivirals, and tricyclic antidepressants (Abaza et al., 2007; Murry et al., 2007).

Table 2

Associations Between Vocal Quality Indices and Executive, and Motor Indices.

	Women with the *FMR1* Premutation					Control Women				
	Mean F0 (Hz)	**Standard deviation of F0 (Hz)**	**Harmonics-to-noise ratio (dB)**	**Jitter %**	**Shimmer %**	**Mean F0 (Hz)**	**Standard deviation of F0 (Hz)**	**Harmonics-to-noise ratio (dB)**	**Jitter %**	**Shimmer %**
Spatial Addition Standard Score	−0.01	0.00	0.21*	−0.16	−0.33*	−0.01	0.00	−0.01	−0.02	−0.24
Hayling Converted Error Score	0.06	0.01	0.13	−0.09	−0.14	0.11	−0.02	0.19	0.05	−0.09
Brown ADD Total Score	0.13	0.00	−0.22	0.25	0.12	0.31*	0.00	−0.04	0.03	−0.09
Functional Tremor Disability Questionnaire	−0.11	0.00	−0.21	0.22	0.14	0.22	−0.01	0.03	0.34	0.12
RAND Health PCS	−0.05	0.10	−0.07	0.00	−0.19	−0.12	0.01	−0.21	0.12	−0.09
NIH Balance Standard Score	−0.22	0.00	−0.13	0.09	−0.13	−0.22	−0.03*	−0.12	0.05	0.25

Note. Standardized β coefficients are presented.

* $p < .050$.