Is Current Fragile X Syndrome Counseling Enough? Expanding the Clinical Phenotype of Fragile X in Premutation And Intermediate Allele Carriers

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IS CURRENT FRAGILE X SYNDROME COUNSELING ENOUGH? EXPANDING THE CLINICAL PHENOTYPE OF FRAGILE X IN PREMUTATION AND INTERMEDIATE ALLELE CARRIERS

by

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DEDICATION

For mummy and papa, for without you and your sacrifices, my dream of being a genetic counselor would have never been discovered, let alone pursued or accomplished.
ACKNOWLEDGEMENTS

I would like to thank my thesis committee members for providing me with guidance and support throughout this process – it has been a challenge, but it feels great to be done!

To the team at USC – thank you for believing in me and helping me on my way to becoming the best counselor that I know I can be. These past two years have provided me with an open and accepting environment which has allowed me to thrive and learn to stand on my own two feet, and I cannot wait to apply what I have learned into helping others. To my classmates – I have absolutely LOVED sharing this experience with you and I look forward to our paths crossing again as colleagues so soon! You made my move away from home so easy and I have loved all of the trivia, the movie nights, the tea, the laughs, and the memories. To my friends and family, I appreciate you for listening about my long days in clinic, the stress regarding exams and nervousness in seeing patients, the tears about patients that I’ll never forget, as well as the successes (e.g. that time where a patient told me that I was a “rising star”) – you have allowed me to share with you an important and integral part of my life and for that, I’ll be forever grateful. Lastly, thank you to my parents and grandparents – there aren’t enough words to express my gratitude; I hope I have made you proud.
ABSTRACT

Fragile X syndrome (FXS) is caused by a triplet repeat expansion on the *FMR1* gene. Individuals with >200 repeats have FXS, while individuals between 45-54 and 55-200 repeats have the *FMR1* intermediate allele and premutation, respectively. FXS is characterized by autism and intellectual disability while the premutation is associated with fragile X-associated tremor/ataxia syndrome (FXTAS) and fragile X-associated primary ovarian insufficiency (FXPOI). However, recent research shows that the premutation may be associated with psychiatric manifestations. Currently, there are no established clinical features associated with the intermediate allele.

This study sought to 1) study knowledge regarding FXTAS, FXPOI, as well as the potential for psychiatric manifestations in individuals with the premutation; 2) study which features, if any, intermediate allele carriers exhibit, and 3) learn which resources are most helpful for FXS. Participants were recruited through online Facebook groups and completed one of two surveys. Results showed that 1) individuals in both groups overestimated their chances for FXS-related disorders; 2) significantly more individuals with the intermediate allele experienced depression/anxiety than expected; and 3) the most helpful resources for learning about FXS were internet websites and conversations with health providers and other individuals with the *FMR1* premutation. These findings reveal that genetic counselors should place more emphasis on the genetics of FXS and its associated phenotypes to both groups and offer both traditional sources of support as well as referral to Facebook groups to facilitate conversations with others in similar situations.
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LIST OF ABBREVIATIONS

ASD ......................................................................................... Autism Spectrum Disorder
ADHD ................................................................................. Attention Deficit Hyperactivity Disorder
CGG ....................................................................................... Cytosine-Guanine-Guanine
DNA ....................................................................................... Deoxyribonucleic Acid
FMRI ....................................................................................... Fragile X Mental Retardation 1
FMRP ....................................................................................... Fragile X Mental Retardation Protein
FXS ......................................................................................... Fragile X Syndrome
FXPOI ................................................................. Fragile X-associated Primary Ovarian Insufficiency
FXTAS ................................................................. Fragile X-associated Tremor/Ataxia Syndrome
RNA ....................................................................................... Ribonucleic Acid
SAD ......................................................................................... Social Anxiety Disorder
CHAPTER 1
BACKGROUND

1.1 FRAGILE X SYNDROME OVERVIEW

Fragile X syndrome (FXS), one of the leading causes of X-linked intellectual disability and autism, affects between 1/4,000 to 1/7,000 males and between 1/8,000 to 1/11,000 females (Cornish, Turk, & Hagerman, 2008; Fu et al., 1991; Hunter et al., 2014; Turner, Webb, Wake & Robinson, 1996). It is caused by an expansion of a cytosine-guanine-guanine (CGG) trinucleotide repeat in the 5’ untranslated region of the Fragile X Mental Retardation 1 (FMR1) gene located on the X chromosome (Verkerk et al., 1991). FXS is inherited in an X-linked manner, meaning that it is transmitted from one generation to the next on the X chromosome. This means that none of the sons of a man with the premutation will inherit said premutation while all of his daughters will inherit the premutation, as males give their only X chromosome to their daughters and their Y chromosome to their sons. Additionally, females with the premutation are at an increased risk to have sons and/or daughters affected by FXS due to maternal anticipation, meaning that the repeat has a likelihood of expanding from a premutation allele to a full mutation when passed from mother to child (Bourgeois et al., 2009; Fu et al., 1991), with larger alleles at increased risk for expansion.

The trinucleotide expansion is located in a region of the DNA that is neither transcribed into RNA nor translated into a protein, so it has no effect on the structure or the function of the FMR1 protein product. Instead, a large enough expansion (>200 CGG
repeats) leads to hypermethylation of an adjacent CpG island and causes silencing of the *FMR1* gene. When this gene is silenced, there is no production of the Fragile X Mental Retardation Protein (FMRP), a product necessary for healthy brain maturation, learning, and memory (Schneider, Hagerman & Hessl, 2009; Sidorov, Auerbach & Bear, 2013). In contrast, having fewer than 200 CGG repeats does not lead to hypermethylation of the nearby CpG island, and thus does not lead to the intellectual disability phenotype. Instead, the symptoms that we see vary from what is observed in traditional FXS and are described in further detail below.

The clinical phenotype of FXS varies depending on the number of CGG repeats observed in an individual, and these are summarized in Table 1.1 (Biancalana, Glaeser, McQuaid, & Steinbach, 2015; Debrey et al., 2016; Usdin et al., 2014). Males and females with greater than 200 CGG repeats are said to have FXS while individuals with 55-200 and 45-54 repeats are said to have the *FMR1* premutation and intermediate allele, respectively. Traditionally, having fewer than 45 CGG repeats lead to no clinical manifestations and is considered phenotypically “normal.”

While having >200 repeats leads to FXS, the clinical presentation between the two sexes can vary. This is because males only have one X chromosome, and a mutated X chromosome in males is sufficient to cause the condition. However, since females have two X chromosomes, one deficient copy of the *FMR1* gene on one chromosome is in some ways “masked” by a working copy on the other chromosome. These females can show anywhere from mild to severe symptoms of the phenotype associated with the full mutation due to a phenomenon called skewed X-inactivation, which means that the ratio of cells containing the X chromosome with the functional copy of the *FMR1* gene compared to the
cells containing the X chromosome with the mutated copy of the \textit{FMRI} gene is not equal. In general, it is thought that 1/3 of females with FXS have no symptoms, 1/3 have learning disabilities, and 1/3 have intellectual disability, but there are no definite or concrete numbers supporting this (Fragile X Syndrome, 2017). In light of all of the ongoing research, it is important to note that the spectrum of severity varies in both males and females, with the latter showing even more clinical variability ranging from mild to severe involvement.

Having $>200$ CGG repeats at the 5’ end of the \textit{FMRI} gene leads to abnormal methylation of a nearby \textit{FMRI} CpG island. These islands are located near the “beginning” of the coding region of the gene, and whether or not this area is methylated determines whether or not the gene is transcribed, or “read” to eventually be made into a protein. This abnormal methylation leads to an absence of \textit{FMRI} transcription and ultimately leads to the phenotype that we see in affected individuals, which is described in further detail below (Sutcliffe et al., 1992). Males with a full mutation (as defined as having $>200$ CGG repeats) show several clinical features, including moderate intellectual disability, and autism spectrum disorder (ASD). Among individuals with FXS, rates for ASD are roughly 60% for males and 14.3% for females (Klusek, Martin, & Losh, 2014). Individuals with both FXS as well as ASD have more severe behavioral problems, such as attention deficit hyperactivity disorder (ADHD), than individuals with FXS alone (Niu et al., 2017). Behavioral manifestations can include social anxiety and withdrawal, language and learning deficits, hyperactivity, aggression, and self-injurious behaviors. Physical features include a large head, prominent forehead and chin, prominent ears, and connective tissue findings such as joint laxity, and macroorchidism (Hagerman & Hagerman, 2002;
Reviewed by Saul & Tarleton, 2012). Due to skewed X-inactivation, females can show some of the same clinical phenotypes observed in full mutation males, although much milder; however, this can vary.

*FMRI* alleles undergo a phenomenon known as anticipation, meaning that the signs and symptoms of the genetic condition tend to be more severe and/or appear at an earlier age as the disorder is passed from one generation to the next (NCI Dictionary). In FXS, the maternally inherited allele is at increased risk for expansion from one generation to the next, and this risk of expansion correlates with the size of the allele (Fu et al., 1991). The risk of a premutation allele expanding to a full mutation is >98% for alleles with >100 CGG repeats (Nolin et al., 2011). Gray zone allele analysis has shown that about 14% of intermediate alleles inherited from the mother are unstable and may expand to the premutation range, but not to the full mutation (Nolin et al., 2011). These numbers are still being researched, but it is important to consider is that many individuals are carriers of the gray zone or premutation allele and do not know until they have children that are affected with one of the associated clinical conditions.

### 1.1 FRAGILE X PREMUTATION

The fragile X premutation is defined as having between 55-200 CGG repeats on the *FMRI* gene (Tassone, Hagerman, & Hagerman, 2014). Individuals with the *FMRI* premutation allele can experience a wide variety of clinical features, and the literature shows that about 1/291 females and 1/855 males have the *FMRI* premutation (Hunter et al., 2014). Historically, individuals with the *FMRI* premutation were not found to be at increased risk for any clinical phenotypes themselves, but rather were advised of the allele expansion risk for their children and grandchildren (Wheeler et al., 2014). Research over
the last twenty years has disproved that initial theory and now, the two widely known associations for individuals with the *FMR1* premutation are fragile X-associated primary ovarian insufficiency (FXPOI) and fragile X-associated tremor/ataxia syndrome (FXTAS). More recent literature shows that, compared to the general population, individuals with the *FMR1* premutation are also at increased risk for immune-mediated disorders, fibromyalgia, hypertension, and migraines (Wheeler et al., 2014). Although the aforementioned symptoms are clinically linked to FXS, the main focus for this study will be to assess knowledge of the widely known FXPOI and FXTAS, and more recently, the psychiatric manifestations such as depression and anxiety that may be correlated with having the *FMR1* premutation (Bourgeois et al., 2011; Hagerman & Hagerman, 2013; López-Mourelo et al., 2017).

### 1.2 Fragile X-Associated Primary Ovarian Insufficiency (FXPOI)

Primary ovarian insufficiency is defined as the loss of ovarian hormonal function at or before the age of 40 and is associated with early onset of menopause (as reviewed by Barasoain et al., 2016), and research shows that approximately 16%-20% of females with the *FMR1* premutation show FXPOI (Rodriguez-Revenga et al., 2009; Schwartz et al., 1994). Menopause is associated with the cessation of menses, and research has shown that individuals with the *FMR1* premutation undergo menopause five years earlier than their counterparts without the premutation at approximately age 40 (Murray et al., 2014), but the mean age for menopause is 51 years old and ranges between 48 and 54 years (as reviewed by Barasoain et al., 2016).

Features of FXPOI include diminished ovarian reserve leading to irregular menses, elevated FSH levels, and reduced fertility (Barasoain et al., 2016). It is important to note
that the size of the premutation as well as the percentage of active X chromosomes with
the mutation has no significant effect on the age at which menopause occurs, and that there
is no increased risk for POI in females who have the full mutation (Murray, Ennis,
MacSwiney, Webb, & Morton, 2000). Although about 1/5 women display the POI
phenotype, it is important for all females with the \textit{FMR1} premutation to be aware of these
risks so that appropriate reproductive options and management can be considered.

1.3 FRAGILE X-ASSOCIATED TREMOR/ATAXIA SYNDROME (FXTAS)

Ten years after the discovery of the \textit{FMR1} gene, FXTAS was first described in
2001 in males with the premutation allele (Hagerman et al., 2001). Research shows that
amongst individuals with the \textit{FMR1} premutation, approximately 45\% of males and
between 8\%-16\% of females are affected with FXTAS (Espinel, Charen, Huddleston,
Visootak, & Sherman, 2016; Rodriguez-Revenga et al., 2009). In comparison to the early
onset of POI in premutation females, FXTAS typically onsets in the sixth decade of life
and older males are at higher risk (Jacquemont et al., 2004). FXTAS is a neurodegenerative
disorder, which is characterized by progressive cerebellar ataxia, with the major two
clinical signs being intention tremor or gait ataxia (as reviewed by Saul & Tarleton, 2012).
Other features include Parkinsonism, autonomic dysfunction and cognitive decline
(Connon & Larner, 2017; Hagerman et al., 2001; Juncos et al., 2011). Individuals may also
have differences in mood, such as irritability and/or anger, or psychiatric changes such as
depression and/or anxiety (Bacalman et al., 2006; Jacquemont et al., 2004). Additionally,
research has shown that men with the \textit{FMR1} premutation and FXTAS may experience
neuropsychiatric symptoms such as somatization, obsessive compulsive, depression,
anxiety, psychoticism, agitation/aggression, apathy/indifference, irritability, and nighttime
behavioral problems (Grigsby et al., 2016). However, these results showed that these psychiatric manifestations may be due to the knowledge of “cognitive and physical dysfunction rather than reflecting psychosis” (Grigsby et al., 2016), and there was no significant increase in psychiatric manifestations in men with the \textit{FMR1} premutation without FXTAS.

FXTAS may present in childhood with seizures or early menopause (Noto, Harrity, Walsh, & Marron, 2016). Some medical problems that are related to FXTAS but occur before its onset include immune-mediated disorders, hypertension, autonomic dysfunction, sleep apnea, hearing loss, and migraines (as reviewed by Hagerman & Hagerman, 2013), and these are more common in women with FXTAS (Coffey et al., 2008; Hagerman & Hagerman, 2013; Wheeler et al., 2014). The incomplete penetrance of FXTAS in individuals with the \textit{FMR1} premutation has been suggested to be due to the presence of other genetic and/or environmental factors that contribute to its manifestation (Hagerman & Hagerman, 2013).

\textbf{1.4 FRAGILE X-ASSOCIATED PSYCHIATRIC MANIFESTATIONS}

While having the \textit{FMR1} premutation allele increases one’s risk for FXTAS in primarily males and FXPOI in females (Cronister et al., 1991; Hagerman et al., 2001), recently there has been research that may show that individuals with the \textit{FMR1} premutation are at increased risk for developing psychiatric disorders such as anxiety and depression. Bourgeois et al. (2009) reported clinical manifestations of psychiatric illness in individuals with the premutation, including cognitive, mood, anxiety, and other psychiatric disorders (e.g. depression and social disorders like schizotypal personality disorder, avoidant personality disorder, and social phobia) (Bourgeois et al., 2009). A 2011 study of 85 men
and women with the fragile X premutation indicated that the lifetime rates of social phobia in individuals with the premutation and without FXTAS were significantly higher than controls (Bourgeois et al., 2011). More recently, research has shown an association between females with the \textit{FMR1} premutation and psychiatric manifestations as well, particularly social anxiety disorder (SAD) and autism spectrum disorder (ASD), (López-Mourelo et al., 2017), with 40% of individuals with a premutation exhibiting depression and/or anxiety (Hagerman & Hagerman, 2013). While individuals with FXTAS are more likely to show behavioral features including depression and/or anxiety, individuals with the premutation without FXTAS are also at risk to show the phenotype, with premutation females being more susceptible to social phobia than premutation males (López-Mourelo et al., 2017). Additionally, an amalgamation of previous research shows that females with the \textit{FMR1} premutation appear to be at increased risk for dementia, hypothyroidism, hypertension, seizures, fibromyalgia, autoimmune diseases, neuropathies, migraines, and postpartum depression (Coffey et al., 2008; Finucane et al., 2012; Hoyos & Thakur, 2017; Wheeler et al., 2014).

Some studies have shown that the development of anxiety disorders in premutation females could be associated with the complications involved with FXPOI, the burden of taking care of a child with intellectual disabilities (for women who have a child affected with FXS), as well as skewed X-inactivation, which could lead to a more apparent FXS phenotype in women (Kenna et al., 2013; Mailick et al., 2014; Roberts et al., 2016). However, a recent study showed that there is a significant increase in psychiatric manifestations in females with the \textit{FMR1} premutation, including those who do not have children affected with FXS, than in controls. In this same study including 24 women with
the premutation and 26 women without it, women with the *FMR1* premutation were shown to have significantly higher rates of social phobia (42.3%) compared to controls (12.5%) (Gossett et al., 2016).

Although the potential association between being a female with the *FMR1* premutation and increased susceptibility to psychiatric conditions has been established since at least the year 2009, the practice guidelines proposed by the National Society of Genetic Counselors (NSGC) does not include counseling for these clinical manifestations due to the fact that these associations do not yet have enough supportive evidence to warrant a change in the clinical guidelines (Finucane et al., 2012). The NSGC uses a stringent evidence-based grading scale to evaluate clinical and research evidence to determine whether there is a significant enough association to be included in a practice guideline (Practice Guidelines Committee, 2016); therefore, this research is exploratory in nature. However, with increasing numbers of patients exhibiting these signs and symptoms, it would be beneficial to know what information, if any, patients are learning about the psychiatric manifestations, and from which sources.

**1.5 FRAGILE X GRAY ZONE – WHAT WE KNOW**

The American College of Medical Genetics (ACMG) defines the intermediate/gray zone as being between 41-54 CGG repeats, but the ACMG laboratory practice committee defines this range as being between 45-54 repeats (Kronquist, Sherman, & Spector, 2008; Maddalena et al., 2001; Monoghan, Lyon, Spector, & American College of Medical Genetics and Genomics, 2013). Additionally, some studies put this number between 40-54 CGG repeats (Tassone et al., 2012). Other sources report the lower range as low as 34 CGG repeats and the higher range at 60 repeats (Hall, 2014; Tassone et al., 2012). The
discrepancy between different laboratories and professional societies exists due to the lack of knowledge about this allele, and Hall summarizes that, “it is not clear if the gray zone should be defined based on the likelihood of expansion in later generations, by associated phenotypes, or by underlying molecular abnormalities” (Hall et al., 2014). Additionally, there is even discrepancy regarding the naming of this range. These include “gray zone,” “intermediate,” “inconclusive,” and “borderline” (Monoghan, Lyon, Spector, & American College of Medical Genetics and Genomics, 2013). These inconsistencies themselves make it difficult to make clinical assertions and require more study.

Contrary to the traditional assertion that intermediate allele carriers show no clinical manifestations, the clinical spectrum FXS may have recently expanded to include gray zone/intermediate allele carriers. It is believed that the incidence of the intermediate/gray zone allele in the general population ranges from 0.3%-2.6%, (As reviewed by Hall, Tassone, Kepitskaya, & Leehey, 2012) and historically, this was thought to have no implications for the individuals themselves, but rather for subsequent generations. However, recent research shows that these individuals may have some clinical manifestations as well, including Parkinsonism features (defined as having two of the following: bradykinesia, resting tremor, rigidity, asymmetric onset), which potentially expands the phenotypic spectrum of FXS (Debrey et al., 2016; Hall, Tassone, Kepitskaya, & Leehey, 2012; Liu, Winarni, Zhang, Tassone, & Hagerman, 2013).

The association of intermediate alleles to the manifestation of primary ovarian insufficiency is currently being disputed, with some authors finding a higher significance of POI in intermediate carriers and others finding no such correlation. In one study of 53 women with primary ovarian insufficiency, 15/106 alleles (14.2%) were between and
including 35 and 53 CGG repeats in the FMR1 gene, compared to 6.5% prevalence in 322 control alleles of individuals in the general population (Bretherick, Fluker, & Robinson, 2005). Another study showed 9/190 individuals (4.7%) had POI, although the repeat sizes ranged from 41-58 CGG repeats (Bodega et al., 2006), and it is important to take into consideration that the upper end of this range falls into the widely described premutation zone (anything over 55 CGG repeats). Pastore and colleagues studied women with a diminished ovarian reserve and found that out of 62 women, 14.5% had between 35-44 CGG repeats in the FMR1 gene, considering that since these results may be overrepresented in this population of women, it is a potential limitation of this study (Pastore et al., 2012). However, recent research completed within the last decade does not support these findings. A study of 366 women with POI showed no significant difference between intermediates and controls, and these findings were replicated by others using similar group sizes (Bennett, Conway, Macpherson, Jacobs, & Murray, 2010; Murray et al., 2014; Voorhuis et al., 2012). Due to the conflicting evidence regarding this association, this present study will not focus on POI in these individuals.

In contrast to the conflicting evidence for the association of intermediate alleles with POI, it is widely supported that the FXTAS phenotype has been identified in both male and female intermediate allele carriers (Hall, Tassone, Kepitskaya, & Leehey, 2012; Liu, Winarni, Zhang, Tassone, & Hagerman, 2013). In these studies, five gray zone individuals were described to have clinical features consistent with a diagnosis of FXTAS. Three of these five individuals were women whose neurological features began in their 50s and 60s and slowly progressed over the course of twenty to thirty years. Of these five individuals, the smallest CGG repeat size was 47 repeats, which is on the lower end of the
gray zone range. A study in 2011 showed that the \textit{FMR1} intermediate allele was associated with Parkinson disease features in women (Hall et al., 2011). These findings were supported by a case study done in 2016 that showed that gray zone carriers exhibited Parkinsonism features such as movement disorders and memory loss (Debrey et al., 2016). Additionally, a study in Iran involving 154 males with Parkinson’s disease and 190 age-matched healthy controls showed that 11/154 males (7.14%) with Parkinson’s disease were found to be carriers of the \textit{FMR1} intermediate allele while 3/190 healthy males (1.57%) were found to be intermediate allele carriers (Entezari, Khaniani, Bahrami, Derakhshan, & Darvish, 2017). While this knowledge does not yet have clinical utility or guidelines indicating whether there is a need to counsel patients about this association, identifying more intermediate allele carriers and observing their clinical phenotypes on a grander scale may help to expand knowledge about the phenotypic spectrum of FXS.

1.6 AVAILABLE RESOURCES

Many online community support resources exist for individuals with FXS as well as for individuals with the \textit{FMR1} premutation. A quick Facebook search reveals a number of groups for individuals with FXS and those who have the premutation, each of which has between a few hundred and a few thousand members, creating a supportive community worldwide. However, it is difficult to find these groups for individuals who are carriers of the intermediate/gray zone allele. Espinel et al. (2016) found that having “family members [familiar with FXS], national and community organizations, research studies, compassionate physicians, and interactions with other individuals with the \textit{FMR1} premutation” facilitated patients’ own healthcare journeys (Espinel, Charen, Huddleston, Visootak, & Sherman, 2016). However, barriers included lack of knowledge about FXS...
among healthcare providers and among the women themselves, shortage of premutation-specific support, and targeted educational materials (Espinel, Charen, Huddleston, Visootak, & Sherman, 2016). In this day and age where social media has the potential to be a significant source of support at one’s fingertips, it is important to learn which groups, if any, are most helpful so genetic counselors may direct their patients there.

A recently published paper by Rocha et al. (2017) showed that individuals are turning to social media (e.g. blogs, Facebook groups, and Twitter) to find and connect with other individuals who are on the same healthcare journeys. Out of 103 individuals who participated in the research, Facebook was the most popular source for support, with 99% of them turning to this medium as a resource (Rocha et al., 2017). Results showed that social media was used to “look for information about their diagnosis or test results (83%), read posts from rare disease groups or organizations (73%), participate in conversations about their diagnosis (67%), and connect with others to find support (58%)” (Rocha et al., 2017). Anecdotal findings of the researcher herself found that mothers of children with FXS often posted about their children’s trials and tribulations, as well as their achievements, to others experiencing the same struggles and accomplishments. They sought out tips on how to have their child sit still for haircuts, asked for recommendations for doctors, and how best to potty-train their children with FXS among other questions that could be answered by others on the same journey. Additionally, individuals asked about guidance regarding their genetic testing results (FXS groups), hope for successful pregnancy based on hormone levels and various reproductive therapies (POI groups) as well as for support regarding declining health (FXTAS groups). These online groups serve as support for parents all over the world and allow them to feel as if they are not alone in
navigating their child’s health and well-being. Having a better idea of which of these groups (and other resources) offer the most support is beneficial to genetic counselors and other healthcare providers alike.

1.7 RATIONALE FOR RESEARCH

Currently, the ACMG recommends carrier screening for FXS to women with a family history of intellectual disability and/or autism, those who are known to have the \textit{FMR1} premutation, as well as those with a family history of FXS (Sherman, Pletcher, & Driscoll, 2005). ACMG does not recommend population carrier screening for FXS. The American College of Obstetricians and Gynecologists (ACOG) published a committee opinion echoing the recommendations of the ACMG but adding that women who have unexplained ovarian insufficiency or failure or an elevated follicle-stimulating hormone level before age 40 years should also get \textit{FMR1} carrier screening (American College of Obstetricians and Gynecologists Committee on Genetics, 2010). They also recommend offering testing and genetic counseling to women who request it, regardless of family history. However, with FXS mutation screening being offered on numerous prenatal screening panels, there is an increase in the uptake of \textit{FMR1} carrier testing making it important to have accurate information on which to base clinical care as well as with which to make an informed decision about whether to pursue screening (Finucane, Lincoln, Bailey, & Martin, 2017).

The literature shows that 1/291 females and 1/855 males have the \textit{FMR1} premutation (Hunter et al., 2014), and having a premutation or intermediate/gray zone allele has the potential to induce anxiety in the client. A study done in Australia showed that women with no family history of FXS who were found to have the \textit{FMR1} premutation
did experience anxiety and stress since they were not expecting to receive an abnormal result during routine prenatal carrier screening (Beard, Amor, Di Pietro, & Archibald, 2016). Additionally, few educational materials are available for patients and providers regarding the clinical spectrum of FXS (Espinel, Charen, Huddleston, Visootak, & Sherman, 2016). Providing appropriate pretest counseling about the risks of carrying one of these alleles as well as its implications for not only the client, but also to her children, is imperative in providing thorough informed consent. While many people are counseled about the risks of developing FXPOI as well as FXTAS, it is not known whether individuals with the FMR1 premutation are aware of the potential risks for psychiatric manifestations such as depression and anxiety. Of course, this is an area of research that is still being developed and more information and data needs to be collected before making this clinical assertion or counseling about it. However, shedding light on this issue would provide genetic counselors with a better understanding with which to approach informed consent and clinical care with their patients.

Both males and females with the FMR1 premutation are at risk for FXTAS and women are at increased risk for FXPOI. Hearing about the risks of these two manifestations alone can be life-altering for individuals who are learning of their premutation status, but recent research shows that the need to counsel about psychiatric manifestations may be necessary. Currently, the guidelines set forth by the NSGC for counseling of FXS involves providing information about FXTAS and FXPOI for individuals who are found to have the FMR1 premutation, but not for the psychiatric manifestations that are now being observed in these individuals (Finucane et al., 2012). Additionally, these guidelines do not include any type of counseling for gray zone allele carriers, highlighting the need to study this topic.
further. Therefore, this study may indicate the need to update the current clinical practice guidelines for FXS.

Initially, individuals in the intermediate allele range were informed that they would not show any clinical manifestations. However, a handful of case reports are showing that intermediate allele carriers are experiencing Parkinsonism features and FXTAS, which brings to attention the importance of identifying more individuals in this gray zone range. Although the clinical manifestations of intermediate allele carriers have not yet been discussed broadly in a clinical context, the responses ascertained from this study may have implications for practice. In light of these recent developments, genetic counselors may need to expand their procedures for informed consent when counseling about prenatal carrier screening panels to include these recent clinical developments and provide thorough pretest counseling. Further elucidating the phenotype in a greater number of individuals would help to establish the more common features that are observed and delegate how better to counsel individuals who are identified as intermediate allele carriers. Additionally, the profession of genetic counseling involves life-long learning and it is important to be aware of new research for not only the purposes of continuing education but also the resultant improvement in patient-centered counseling.

A previous thesis project done by a Master’s Student in Genetic Counseling at Brandeis University explored individuals with the $FMR1$ premutation knowledge of the associated clinical phenotypes by using survey methodology. The survey included awareness of FXPOI and FXTAS but not any of the psychiatric manifestations that may be associated with having a premutation allele in $FMR1$. Additionally, the sample size was
small (n=43) and it did not include intermediate/gray zone allele carriers (Metterville, 2009).

This study aims to expand upon that research by gauging premutation and intermediate allele carriers’ knowledge of not only their risks for FXTAS and FXPOI, but also the psychiatric symptoms that are now thought to be associated with FXS. Secondly, this study seeks to expand knowledge about intermediate allele carriers of the \( FMR1 \) gene. There exist some case reports of their clinical manifestations, but there needs to be more documentation of their clinical features to expand the phenotype of this intermediate/gray zone range. Lastly, community support resources for individuals with FXS and those who are have the \( FMR1 \) premutation are in abundance, but there are no resources easily located for intermediate allele carriers. Identifying resources that would be most useful to the patients would help to provide genetic counselors with better tools with which to direct patients for support, especially since research is showing that psychiatric symptoms may be present in individuals with the \( FMR1 \) premutation and referral to psychological services may be warranted.
Table 1.1 Molecular Classification and Clinical Manifestations Based on # of CGG Repeats in *FMR1*

<table>
<thead>
<tr>
<th>Allele Classification</th>
<th># of Repeats</th>
<th>Clinical Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unaffected</td>
<td>&lt;45</td>
<td>No clinical features</td>
</tr>
<tr>
<td>Intermediate/</td>
<td>46-54</td>
<td>Potential Parkinsonism and neuropsychiatric features (later in life)</td>
</tr>
<tr>
<td>Gray Zone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premutation</td>
<td>55-200</td>
<td>Fragile X-associated Tremor/Ataxia Syndrome (FXTAS), Fragile X-associated Primary Ovarian Insufficiency (FXPOI) in females, potential for psychiatric manifestations (i.e. depression, anxiety)</td>
</tr>
<tr>
<td>Full Mutation</td>
<td>&gt;200</td>
<td>Intellectual disability, autism, facial dysmorphism, ADHD</td>
</tr>
</tbody>
</table>
CHAPTER 2

IS CURRENT FRAGILE X SYNDROME COUNSELING ENOUGH? EXPANDING THE CLINICAL PHENOTYPE OF FRAGILE X IN PREMUTATION AND INTERMEDIATE ALLELE CARRIERS

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ABSTRACT

Fragile X syndrome (FXS) is caused by a triplet repeat expansion on the FMR1 gene. Individuals with >200 repeats have FXS, while individuals between 45-54 and 55-200 repeats have the FMR1 intermediate allele and premutation, respectively. FXS is characterized by autism and intellectual disability while the premutation is associated with Fragile X-associated tremor/ataxia syndrome (FXTAS) and Fragile X-associated primary ovarian insufficiency (FXPOI). However, recent research shows that the premutation may be associated with psychiatric manifestations. Currently, there are no established clinical features associated with the intermediate allele.

This study sought to 1) study knowledge regarding FXTAS, FXPOI, as well as the potential for psychiatric manifestations in individuals with the premutation; 2) study which features, if any, intermediate allele carriers exhibit, and 3) learn which resources are most helpful for FXS. Participants were recruited through online Facebook groups and completed one of two surveys. Results showed that 1) individuals in both groups overestimated their chances for FXS-related disorders; 2) significantly more individuals with the intermediate allele experienced depression/anxiety than expected; and 3) the most helpful resources for learning about FXS were internet websites and conversations with health providers and other individuals with the FMR1 premutation. These findings reveal that genetic counselors should place more emphasis on the genetics of FXS and its associated phenotypes to both groups and offer both traditional sources of support as well as referral to Facebook groups to facilitate conversations with others in similar situations.

Key words: Fragile X syndrome, premutation, intermediate/gray zone, FXTAS, FXPOI, psychiatric, resources
INTRODUCTION

Fragile X syndrome (FXS), one of the leading causes of X-linked intellectual disability and autism, affects between 1/4,000 to 1/7,000 males and between 1/8,000 to 1/11,000 females, (Cornish, Turk, & Hagerman, 2008; Fu et al., 1991; Hunter et al., 2014; Turner, Webb, Wake & Robinson., 1996) and is caused by an expansion of a cytosine-guanine-guanine (CGG) trinucleotide repeat in the 5’ untranslated region of the Fragile X Mental Retardation 1 (FMR1) gene (Verkerk et al., 1991). The clinical phenotype of FXS varies depending on the number of CGG repeats observed in an individual, and these are summarized in Table 2.1 (Biancalana, Glaeser, McQuaid, & Steinbach, 2015; Debrey et al., 2016; Usdin et al., 2014). Males with greater than 200 CGG units have fragile X syndrome and females with greater than 200 CGG repeats are considered carriers of the full mutation. Any individual who has between 55 and 200 CGG units is considered to have the FMR1 premutation and having 45-54 repeats puts an individual in the intermediate/gray zone allele range (Sherman, Pletcher, & Driscoll, 2005; Tassone et al., 2012). Traditionally, having fewer than 45 CGG units leads to no clinical manifestations and is considered phenotypically “normal.”

Males with a full mutation show several clinical features, including moderate intellectual disability and autism spectrum disorder (ASD). Behavioral manifestations can include social anxiety and withdrawal, language and learning deficits, hyperactivity, aggression, and self-injurious behaviors. Physical features include a large head, prominent forehead and chin, prominent ears, and connective tissue findings such as joint laxity, and macroorchidism (Hagerman & Hagerman, 2002; Reviewed by Saul & Tarleton, 2012). Due
to skewed X-inactivation, females can show some of the same clinical phenotypes observed in full mutation males, although much milder; however, this can vary.

Individuals with the \textit{FMR1} premutation allele can experience a wide variety of clinical features, and the literature shows that about 1/209 females and 1/430 males have the \textit{FMR1} premutation (As reviewed by Hall, 2014). The two widely known associations for the \textit{FMR1} premutation are fragile X-associated primary ovarian insufficiency (FXPOI) and fragile X-associated tremor/ataxia syndrome (FXTAS). More recent literature shows that, compared to the general population, premutation carriers are also at increased risk for immune-mediated disorders, fibromyalgia, hypertension, and migraines (Wheeler et al., 2014). Although the aforementioned symptoms are clinically linked to fragile X syndrome, the main focus of this study will be assessing knowledge of the widely known FXPOI and FXTAS, and more recently, the psychiatric manifestations such as depression and anxiety that may be correlated with having the \textit{FMR1} premutation allele (Bourgeois et al., 2011; Hagerman & Hagerman, 2013; López-Mourelo et al., 2017).

While having the \textit{FMR1} premutation allele increases one’s risk for fragile X tremor/ataxia syndrome (FXTAS) in primarily males (but also females) and fragile X-associated primary ovarian insufficiency (FXPOI) in females (Cronister et al., 1991; Hagerman et al., 2001), recently there has been research that may show that premutation carriers are at increased risk for developing psychiatric disorders such as anxiety and depression. Bourgeois et al. (2009) reported clinical manifestations of psychiatric illness in premutation carriers, including cognitive, mood, anxiety, and other psychiatric disorders. While individuals with FXTAS are more likely to show behavioral features including depression and/or anxiety, individuals with the premutation without FXTAS are
also at risk to show the phenotype, with premutation females being more susceptible to social phobia than premutation males (López-Mourelo et al., 2017). Additionally, an amalgamation of previous research shows that females with the \textit{FMR1} premutation allele appear to be at increased risk for dementia, hypothyroidism, hypertension, seizures, fibromyalgia, autoimmune diseases, neuropathies, migraines, and postpartum depression (Coffey et al., 2008; Finucane et al., 2012; Hoyos & Thakur, 2017; Wheeler et al., 2014).

Although the potential association between being a female with the \textit{FMR1} premutation and increased susceptibility to psychiatric conditions has been established since at least the year 2009, the practice guidelines proposed by the National Society of Genetic Counselors (NSGC) does not include counseling for these clinical manifestations due to the fact that these associations have not yet been well established (Finucane et al., 2012). The NSGC uses a stringent evidence-based grading scale to evaluate clinical and research evidence to determine whether there is a significant enough association to be included in a practice guideline (Practice Guidelines Committee, 2016); therefore, this research is exploratory in nature. However, with increasing numbers of patients exhibiting these signs and symptoms, it would be beneficial to know what information, if any, patients are learning about the psychiatric manifestations, and from which sources.

Contrary to the traditional assertion that intermediate allele carriers show no clinical manifestations, the clinical spectrum of fragile X syndrome may have recently expanded to include gray zone/intermediate allele carriers. The association of intermediate alleles to the manifestation of primary ovarian insufficiency is currently being disputed, with some authors finding a higher significance of POI in intermediate carriers and others finding no
such correlation. Due to the conflicting evidence regarding this association, this study will not focus on POI in these individuals.

In contrast to the conflicting evidence for the association of intermediate alleles with POI, recent research has shown that the FXTAS phenotype and some Parkinsonism features (Defined as having two of the following: bradykinesia, resting tremor, rigidity, asymmetric onset) has been identified in both male and female intermediate allele carriers (Hall, Tassone, Kepitskaya, & Leehey, 2012; Liu, Winarni, Zhang, Tassone, & Hagerman, 2013). While this knowledge does not yet have clinical utility or guidelines indicating whether there is a need to counsel about this association, identifying more intermediate allele carriers and observing their clinical phenotypes on a grander scale may help to expand knowledge about the phenotypic spectrum of FXS.

Many resources exist for individuals with fragile X syndrome as well as for individuals with the *FMR1* premutation. However, it is difficult to find these groups for individuals who are carriers of the intermediate allele. In this day and age where social media has the potential to be a significant source of support at one’s fingertips, it is important to learn which groups, if any, are most helpful so genetic counselors and other health care providers may direct their patients there. These groups serve as support for parents all over the world and allow them to feel as if they are not alone in navigating their child’s health and well-being.

**MATERIALS AND METHODS**

**2.1 PARTICIPANTS**

Males and females with either the *FMR1* premutation or carriers of the *FMR1* intermediate/gray zone allele were invited to participate in this study. Participants had to
be over 18 years of age and have a formal diagnosis of having one of these alleles through means of previous genetic testing. Only English-speaking participants were included in the study due to limited resources available for interpretation from English to other languages. Additionally, an 80% questionnaire completion rate was required for inclusion in data analysis.

*Individuals with the FMR1 Intermediate/Gray Zone Allele*

Thirty-nine \textit{FMR1} intermediate allele carriers participated in the study, of which 30 completed 80% of the questionnaire, thus rendering inclusion into data analysis. Of these 30 participants, 3/30 (10%) were male, 21/30 (70%) were female, and 6/30 (20%) chose not to answer. The participants’ ages ranged from 27 to 77 years old. Regarding country of residence, 15/30 (50%) lived in the United States and 15/30 (50%) lived abroad (e.g. United Kingdom, England, Ireland). Their education levels ranged from less than high school (1/30; 0.03%) to graduate or professional degrees (6/30; 20%), of which five had master’s degrees and one individual had a medical degree. Overall, 20/30 (67%) of participants had some college education or higher (Table 2.2).

*Individuals with the FMR1 Premutation*

Two hundred forty-seven individuals with the \textit{FMR1} premutation started the questionnaire, out of which 184 participants had 80% completion and subsequent inclusion into further analysis. Of those excluded, 12 of them started the survey and said that they have a grandson with FXS, but that their own mutation status was unknown. The remaining excluded participants marked that they had the premutation but completed none of the other items on the questionnaire. Of these participants, 3/184 (1.6%) were male, 124/184 (67.4%) were female, and 57/184 (31%) chose not to answer. Their ages ranged from 26 to 69 years.
old. Regarding country of residence, 66/184 (35.9%) live in the United States and 62/184 (33.7%) live abroad (e.g. Australia, Belgium, Canada, England, France, Harris, India, Ireland, Netherlands, New Zealand, Qatar, Scotland, South Africa, Spain, Switzerland, and Wales). Their education levels ranged from less than high school (2/184; 1.1%) to graduate or professional degrees (57/184; 31%), of which 13 held Bachelor’s degrees, 29 had Master’s degrees, two had doctoral degrees, three held law degrees, and four held medical degrees. Interestingly, one of the participants had a master’s degree in Genetic Counseling. Overall, 114/184 (62%) of participants had some college education or higher (Table 2.2).

2.3 INSTRUMENTATION

For purposes of recruitment, an announcement (See Appendix A) was created to be posted onto various social media sites. Along with this, an online questionnaire that was programmed using skip logic was developed through SurveyMonkey.com. Interested participants were asked to complete one of two web-based questionnaires – one for individuals with the FMR1 premutation and another for intermediate/gray zone allele carriers (See Appendices B and C, respectively). Use of an online survey kept participant answers anonymous, and no identifying information was collected from the questionnaire. All data was stored on a password protected website (SurveyMonkey.com) as well as a password protected computer. Additionally, a debriefing form was included at the end of the survey due to the fact that some questions may be perceived as personal and/or sensitive, and a list of both domestic and international psychological support resources was included (Appendix D).

Data was collected using survey methodology and responses were measured using two similar, but separate questionnaires. These measures were modified from an
unpublished thesis done by a student at Brandeis University in 2009 (Metterville, 2009). The original questions were altered to fit the needs of this study to reflect the type of data that this study sought to collect (e.g. some of the questions using “yes, no, or unsure” as answer choices were changed instead to a Likert scale evaluating percentage of risk, and a separate section was created to evaluate the potential psychiatric manifestations associated with FXS).

Both questionnaires contained the same six demographic questions as well as the same questions regarding general knowledge about FXS. Quantitative questions were asked to assess categorical information about the participants, such as gender, age, country of residence, and education level of the participants. Each questionnaire also contained questions regarding the year and reason for fragile X testing, which individual in the family (if anyone) has FXS, what they were told about their result and implications, and the resource where the most useful information about FXS was obtained. Only the premutation questionnaire contained questions specific to fragile X-associated tremor/ataxia syndrome (FXTAS), fragile X-associated primary ovarian insufficiency (FXPOI), and the newly developed questions regarding the potential psychiatric manifestations that may be associated with FXS. The questions for gray zone carriers were created in a similar format to the existing survey questions being used for individuals with the FMR1 premutation but instead asked open-ended questions assessing which, if any, symptoms these patients were experiencing, whether they would benefit from an online support group, and whether his or her result has affected reproductive decision-making. Both questionnaires contained a mix of open and closed ended questions which included multiple choice questions, select all that apply, and evaluation of percentage of risk using a Likert scale.
2.2 PROCEDURES

Recruitment for this study occurred from September 1, 2017 to January 20, 2018 using Dillman’s Internet Survey Design (Dillman, Smyth, & Christian, 2014). Recruitment notices were made weekly in September, biweekly in October, and once a month in November, December, and January. An announcement (Appendix A) about the research study was posted on Facebook on various fragile X syndrome (FXS) support groups, which provided a brief introduction, invitation, and link to the questionnaire on SurveyMonkey.com. Permission was obtained from representatives of each group prior to posting of the announcement. The various Facebook support groups in which the notice was posted included the following: Fragile X syndrome, Girls with Fragile X syndrome, Fragile X Female Carrier Symptoms, Fragile X Society, Fragile X, Fragile X Society – India, Fantastically Fragile X – The FX Brag Room, as well as the Facebook groups of both local and international fragile X foundations including but not limited to, the FRAXA Research Foundation, Fragile X Research Foundation of Canada, the Facebook group of the Fragile X support group in the Netherlands, “Fragile x vereniging Nederland”, Premature Ovarian Failure - Primary Ovarian Insufficiency, and the National Ataxia Foundation.

The invitation described the study to participants, provided the investigators’ contact information, and allowed participants to access the study. Potential participants were then able to decide whether they wanted to continue with the questionnaire, which was estimated to take about 20 minutes to complete. Consent was assumed upon clicking “I agree” on the introductory page of the survey and subsequent survey completion. Participation in this study was voluntary; participants were not compensated to participate.
and had the option to withdraw from the study at any time. Participants were given the option to include their email address to be contacted once study results were ready. Since demographic information was collected at the end of the survey, failure to complete the demographic section resulted in exclusion from data analysis.

2.4 DATA ANALYSIS

The methodology for this study was quantitative and qualitative. SurveyMonkey.com software was used to collect the data, and data collection was completed by the end of January 2018. Data was analyzed in February 2018, where quantitative analysis was done on the questionnaire items, and thematic analysis methods grounded in theory were utilized to identify themes within open-ended responses. Demographic information was used to determine descriptive statistics for each category within each population and frequencies were calculated for the items on the questionnaire regarding knowledge about FXS and its associated phenotypes. An analysis using G*Power predicted that a minimum of 28 participants in each group (i.e., individuals with the FMR1 premutation and intermediate allele/gray zone carriers) would be needed in order to detect statistically significant differences at α=.05, power (1 - β) of .95, and an effect size of .50.

RESULTS

2.5 FMR1 GRAY ZONE

Testing for and Counseling about FXS

Of 30 total participants or individuals with the intermediate allele, 12/30 (40%) said that they underwent FXS testing because they have a child or multiple children with FXS, 3/30 (10%) had a family history of FXS, 2/30 (6.67%) have a family member with the
A total of 6/30 (20%) said “other,” and 2/30 (6.7%) were unsure about their reasons for testing (Figure 2.1). Of those who stated “other,” one of the individuals had testing because she herself had POF, one individual was researching FXS and noticed the pattern of FXS in her family to which her geneticist agreed to testing her, one of the individuals was responding on behalf of his or her son who is a carrier of the intermediate/gray zone allele, two individuals had children with ASD which then spurred their own testing, and one individual was diagnosed via an amniocentesis that his or her mother had which revealed a finding in the gray zone range in the patient.

Many of the participants had family members diagnosed with FXS, and most of these individuals had multiple affected family members. Of the thirty individuals with the FMR1 intermediate/gray zone allele, 14/30 (46.7%) of individuals said that they had son(s) with FXS, 7/30 (23.3%) had affected daughters, 2/30 (6.7%) had affected brothers, 1/30 (3.3%) had an affected sister, 1/30 (3.3%) said that his or her grandsons were affected, 1/30 (3.3%) had an affected cousin, and 1/30 (3.3%) had an affected wife. Of the participants, 5/30 (16.7%) had no one in his or her family affected with FXS, and 3/30 (10%) of individuals stated that they had other family members with the FMR1 premutation.

Regarding when the participants were diagnosed with being an intermediate/gray zone allele carrier, 26/30 (86.7%) were diagnosed between 1991-2017, 1/30 (3.3%) was diagnosed before 1991, and 3/30 (10%) were unsure (Figure 2.1). Of those that were diagnosed between 1991-2017, 11/26 (42.3%) were diagnosed within the last five years. At the time that these individuals were found to be intermediate/gray zone allele carriers,
24/30 (80%) stated that they had received genetic counseling, 3/30 (10%) said that they had not, and 3/30 (10%) were unsure (Figure 2.1).

Of the participants, 17/30 (56.7%) individuals had a formal discussion about FXS with their healthcare provider between 1991-2017, 1/30 (3.3%) had a discussion before 1990, 4/30 (13.3%) were unsure, and 8/30 (26.7%) said that they have never had a formal discussion about FXS with their healthcare provider (Figure 2.1). Of those that had a formal discussion between 1991-2017, 7/17 (41.2%) had it within the last five years. Of the discussions and of the information received, 7/30 (23.3%) were told that there were no implications for their own health 3/30 (10%) of individuals were referred to someone else (either a genetic counselor or another healthcare provider), 5/30 (16.7%) received “very little” or no information, and 1/30 (3.3%) did not remember. Regarding the information that they received, 1/30 (3.3%) individual said that he/she was told that her baby “could be mentally retarded,” 3/30 (10%) of individuals said that it “may be associated with POF” and/or “early menopause,” of which one of those individuals did in fact have POF, and 4/30 (13.3%) said that their result “may have an impact on [his or her] grandchildren” and/or “[his or her] grandchildren may have full-blown FXS.” Of the participants, 1/30 (3.3%) participant said that he or she was told to “inform [his or her] daughter.”

**Knowledge and Education about FXS**

There were two general knowledge questions about FXS presented to both groups, and these results can be found in Figures 2.2 and 2.3. Pertaining answers to the question, “Who has the greatest chance of inheriting a fragile X premutation,” only 7/30 (23.3%) FMR1 intermediate/gray zone allele carriers answered the question correctly (Figure 2.3A), with the answer being “Daughter of a male premutation carrier.” Of the FMR1
intermediate/gray zone allele carriers, 8/30 (26.7%) of individuals responded, “son of a female premutation carrier,” 3/30 (10%) responded “daughter of a female premutation carrier, 5/30 (16.7%) of individuals stated that they “all have the same chance,” and 7/30 (23.3%) stated that they were either unsure or did not answer the question (Figure 2.2). Regarding the question, “Who has the greatest chance of having a child with fragile X syndrome,” 13/30 (43.3%) individuals answered the question correctly (Figure 2.3B), the answer being “female premutation carrier.” 2/30 (6.7%) individuals responded “male premutation carrier, 6/30 (20%) said “both [males and females with the FMR1 premutation] have the same chance of having a child with FXS,” and 9/30 (30%) of individuals were either unsure or did not answer the question (Figure 2.2).

We surveyed participants’ knowledge about the chances of an individual with the FMR1 intermediate/gray zone allele developing conditions seen in FXS as well as in the general population, with the pre-existing knowledge that there are no true established clinical features associated with carrying the FMR1 intermediate/gray zone allele. The chances of developing these conditions were evaluated using a Likert scale, and participants were asked about conditions related to FXS as well as those that are not. Conditions unrelated to FXS and its associated phenotypes included things like heart disease, kidney problems, uterine fibroids, diabetes, gastric reflux, and glaucoma. Conditions related to or potentially associated with FXS and its associated phenotypes included early menopause, social anxiety, depression, and tremor/ataxia. Of 30 total participants, 17-22 individuals answered these questions, and results will be based on this subgroup. These findings are summarized in Figure 2.4. Results showed that for every condition, there was at least one person who stated that there was a 0% chance of
developing the phenotype. Additionally, at least one individual stated that the maximum chance for developing each condition was 50% or higher, with the maximum numbers being 75% chance of developing early menopause, 81% chance of developing gastric reflux, 85% chance for tremor/ataxia, 90% chance of developing social anxiety, and a 90% chance for depression. The mean responses for the chance of developing the following conditions which are unrelated to FXS or its associated phenotypes were as follows: heart disease (15.8%), kidney problems (14.6%), uterine fibroids (21.3%), diabetes (18.4%), gastric reflux (27.8%), and glaucoma (18.9%). The mean responses for the chance of developing the following conditions which are related to FXS or thought to be related to FXS or its associated phenotypes were as follows: early menopause (44%), social anxiety (48%), depression (46.1%), and tremor/ataxia (37.5%). These results indicate that individuals did state that there was a higher chance of developing conditions which are related to FXS and its associated phenotypes than those that are not.

**FXS and Reproductive Decision-Making**

The results regarding the impact of the diagnosis on reproductive decision-making can be found in Figure 2.5A. Of the participants, 13/30 (43.3%) of participants said that his or her result did not affect their reproductive decisions. However, 4/30 (13.3%) of participants said that his or her *FMR1* result led to the use of reproductive technologies, 8/30 (26.7%) stated that they chose to not have more children, and 5/30 (16.7%) did not answer the question.

**Personal Clinical Experiences**

We surveyed whether individuals with the intermediate/gray zone allele experienced any symptoms themselves, and results can be found in Figure 2.6. Of the
individuals with the \textit{FMR1} intermediate/gray zone allele, 4/30 (13.3\%) have tremors, 1/30 (3.3\%) has ataxia, 1/30 (3.3\%) has gait abnormalities, 3/30 (10\%) have neuropathy, 0/30 (0\%) have experienced Parkinsonism features (Defined as having two of the following: bradykinesia, resting tremor, rigidity, asymmetric onset), 6/30 (20\%) have experienced memory loss, 18/30 (60\%) have anxiety, and 14/30 (46.7\%) have depression. Additionally, only 2/30 (6.7\%) of individuals said that there is someone in his or her family who has a formal diagnosis of Parkinson disease, and these individuals included a grandfather and a great-grandfather.

\textit{Utilization of Support}

Of the individuals with the \textit{FMR1} intermediate/gray zone allele, 24/30 (80\%) individuals stated that they would be “likely” or “very likely” to access an online support group for \textit{FMR1} intermediate/gray zone allele carriers (Figure 2.7). Of the participants, 11/30 (36.7\%) of participants stated that they would use a support group to “not feel alone” and to be able to “compare stories,” with one individual stating:

I feel like having children with fragile X is a lonely place to be and having people to compare stories to or for advice and somebody to understand where you’re coming from is something that would carry a high value.

Of the participants, 5/30 (16.7\%) of individuals stated that they would use a support group to ask questions, 5/30 (16.7\%) would use it to give information, and 5/30 (16.7\%) said that they would use it to ask for advice. 3/30 (10\%) of individuals listed that a support group would help them to receive up-to-date info and other available resources.
2.6 FMR1 PREMUTATION

Testing for and Counseling about FXS

Of 184 total participants with the FMR1 premutation, 107/184 (58.2%) said that they underwent FXS testing because they have a child or multiple children with FXS, 24/184 (13%) had a family history of FXS, 17/184 (9.2%) have a family member with the FMR1 premutation, 22/184 (12%) did it as part of prenatal carrier screening, 9/184 (2.7%) said “other,” and 5/184 (2.7%) replied that they had never been tested (Figure 2.1). Of those who stated “other,” many of those individuals had FXS testing as part of their infertility/POF work-up, and one individual had it done for displaying Parkinsonism features.

Many of the participants had family members who have been diagnosed with FXS, and similarly to the pattern that was seen in the intermediate/gray zone allele carriers, many of these individuals (65/184 or 35.3%) had more than one family member diagnosed with FXS in their family. Of these 184 individuals with the FMR1 premutation, 106/184 (57.6%) of individuals stated that they had a child or multiple children with FXS, 15/184 (8.2%) had affected siblings, 26/184 (14.1%) had nieces or nephews with FXS, 8/184 (4.3%) had affected cousins, 11/184 (6.0%) had affected grandchildren, 3/184 (1.6%) had affected parents, 15/184 (8.2%) had distant relatives with FXS, and 17/184 (9.2%) had no one in his or her family diagnosed with FXS.

Regarding when the participants were diagnosed with having the FMR1 premutation, 171/184 (92.9%) were diagnosed between 1991-2017, 11/184 (6%) were diagnosed before 1991, and 2/184 (1.1%) were unsure (Figure 2.1). Of those that were diagnosed between 1991-2017, 79/171 (46.2%) were diagnosed within the last five years.
At the time that these individuals were found to have the \textit{FMR1} premutation, 116/184 (63\%) stated that they had received genetic counseling, 60/184 (32.6\%) said that they had not, and 8/184 (4.3\%) were unsure (Figure 2.1).

Of the participants with the \textit{FMR1} premutation, 128/184 (69.6\%) individuals had a formal discussion about FXS with their healthcare provider between 1991-2017, 3/184 (1.6\%) individuals had a discussion before 1990, 9/184 (4.9\%) were unsure, and 44/184 (23.9\%) said that they have never had a formal discussion about FXS with their healthcare provider (Figure 2.1). Of those that had a formal discussion between 1991-2017, 84/128 (65.6\%) had it within the last five years. Out of the individuals with the \textit{FMR1} premutation, one hundred forty-nine individuals gave additional detail into what information was received from his or her healthcare provider. Of the discussions that were had and information that was received, 59/149 (39.6\%) received “very little” or no information, 45/149 (32.9\%) were counseled about FXTAS and FXPOI, 22/149 (14.8\%) received information about reproductive options/chance of having a child with FXS, 20/149 (13.4\%) were referred to someone else, either a genetic counselor or another specialist, and 8/149 (5.4\%) received resources or a print-out. Interestingly, 2/149 (1.3\%) of individuals were informed about anxiety and depression, 2/149 (1.43\%) of participants were told that they “were not affected” and only 1/149 (0.5\%) individual stated that he/she was told to inform family members to facilitate their testing. One individual quoted that he or she was told “this [result] most certainly could not affect my mental health.”

\textit{Knowledge and Education about FXS}

There were two general knowledge questions about FXS that were presented to both groups and results can be seen in Figures 2.2 and 2.3. Pertaining answers to the
question, “Who has the greatest chance of inheriting a fragile X premutation,” 93/184 (50.5%) of individuals with the FMR1 premutation answered the question correctly (Figure 2.3C), with the answer being “Daughter of a male premutation carrier.” Of the individuals with the FMR1 premutation, 34/184 (18.5%) of individuals responded, “son of a female premutation carrier,” 13/184 (7.1%) responded “daughter of a female premutation carrier, 31/184 (16.8%) of individuals stated that they “all have the same chance,” and 9/184 (4.9%) stated that they were unsure (Figure 2.2). Regarding the question, “Who has the greatest chance of having a child with fragile X syndrome,” 117/184 (63.6%) individuals with the FMR1 premutation answered the question correctly (Figure 2.3D), the answer being “female premutation carrier.” Of these participants, 5/184 (2.7%) responded “male premutation carrier, 52/184 (28.3%) said “both [males and females with the FMR1 premutation] have the same chance of having a child with FXS,” and 10/184 (5.4%) participants were either unsure (Figure 2.2).

We surveyed participants’ knowledge about the chances of an individual with the FMR1 premutation developing conditions seen in FXS as well as in the general population. The chances of developing these conditions were evaluated using a Likert scale (0% – 100%), and participants were asked about conditions that are related to FXS as well as those that are not. Questions were phrased as such: “What is the likelihood [0% – 100%] of a fragile X [premutation or intermediate/gray zone allele] causing _____ in an individual with the FMR1 [premutation or intermediate/gray zone allele]?” Conditions unrelated to FXS and its associated phenotypes included things like heart disease, kidney problems, uterine fibroids, diabetes, gastric reflux, and glaucoma. Conditions that were related to or potentially associated with FXS and its associated phenotypes included early menopause,
social anxiety, depression, and tremor/ataxia. Of one hundred eighty-four total participants, 104-158 individuals answered these questions, and results will be based on this subgroup. These findings can be seen summarized in Figure 2.4. Results showed that for every condition except for tremor/ataxia, there was at least one person who stated that there was a 0% chance of developing the phenotype. The lowest risk for tremor/ataxia was listed to be 6%, meaning that there were no participants who thought there was a 0% chance of developing the condition. Additionally, at least one individual stated that the maximum chance for developing each condition was 95% or higher. The mean responses for the chance of developing the following conditions unrelated to FXS or its associated phenotypes were as follows: heart disease (26.3%), kidney problems (21%), uterine fibroids (30.2%), diabetes (21.3%), gastric reflux (33.6%), and glaucoma (21.7%). The mean responses for the chance of developing the following conditions related to FXS or thought to be related to FXS or its associated phenotypes were as follows: early menopause (57%), social anxiety (63.8%), depression (60.2%), and tremor/ataxia (51.9%).

**FXS and Reproductive Decision-Making**

Of 184 individuals who elaborated on his or her result and its effect on reproductive decision-making, 60/184 (32.6%) said that his or her result did not affect their reproductive decisions or that they had already finished having children when they received the result. Of those who responded that their reproductive decision did not change, 5/60 (12%) said that they had children the natural way knowing that they could pass on their premutation. Additionally, 20/184 (10.9%) said that they did not know about their premutation when they had children, and 5/20 (25%) of those individuals said that their reproductive decisions would have changed had they known about his or her result prior to having children. One
individual who was currently pregnant said, “if [sic] my second boy has a full mutation I will feel awful for not catching it earlier because I would not [have] had him.” Of the participants with the *FMR1* premutation, 38/184 (20.7%) elected to not have more children, and 29/184 (15.8%) of individuals said that they sought the help of reproductive technologies. Some individuals (5/184 or 2.7%) were unable to have children because they had already undergone menopause when they received their results, 8/184 (4.3%) of participants sought out prenatal diagnosis with or without termination, and 9/184 (4.8%) stated that their reproductive decisions changed but did not specify how. Of the individuals with the *FMR1* premutation, 15/184 (8.2%) were either unsure or did not answer the question (Figure 2.5B).

*Suggestions for Genetic Counseling*

We asked individuals for suggestions for genetic counseling for each of the various phenotypes associated with having the *FMR1* premutation, such as FXTAS and FXPOI, but also for the potential psychiatric manifestations sometimes experienced by these individuals and some common themes emerged. In general, individuals asked for follow-up appointments to receive the information in bits and pieces, as it is often too much to handle all at once. Additionally, they asked for referral to support resources and most importantly, they asked for honesty, kindness, compassion, and empathy, as there were individuals who were prepared for “doom and gloom” and given a negative outlook on the diagnosis as well as for the future. One individual said, “don’t [sic] say that everything is going to be negative and life is going to be a struggle,” and another said, “I think it is important to be compassionate yet fully honest with a person receiving a diagnosis. Watching my sister deal with FXPOI while trying to get pregnant was very hard.”
In terms of the individual associations, for the potential associated psychiatric manifestations, one respondent said, “ask [sic] if they have any psychiatric conditions, explain that it is a symptom of this condition and the reason for their trouble with it this whole time, and offer referrals for psychiatric/therapeutic evaluations.” Another said

[The] genetic counselor should immediately point out the rates of depression, anxiety, etc. and ask the person if they’ve experienced this, [and then] point out that [the] diagnosis itself can bring on stress that can exacerbate or even cause these symptoms to arise, and that there are medications that can help.

For FXPOI, individuals asked to be referred to a reproductive endocrinologist or other specialist and to cover some basics about the reproductive options that are available. A few participants asked for spouses to be included into the conversation with one saying “include spouses or significant others in the conversations. Don’t sugarcoat anything. Make the person aware that this is real.” Additionally, one individual stated that it is “very important to emphasize that this can have unpredictable ‘relapses’ so that if they do not want to get pregnant, they should still use contraception until full menopause.” Regarding counseling for FXTAS, individuals said that they would want to learn more about the full trajectory of the deterioration involved with having FXTAS as well as information on what are some of the warning signs.

**Fragile X-Associated Primary Ovarian Insufficiency (FXPOI)**

We asked two knowledge questions about FXPOI which can affect individuals with the *FMR1* premutation. The first question asked the participants to choose all features associated with FXPOI. Of the individuals with the *FMR1* premutation, 108/184 (58.7%) chose “menstrual cycle irregularities,” 100/184 (54.3%) chose “hormone fluctuations,”
116/184 (63%) chose “decreased fertility,” 135/184 (73.4%) chose “early onset menopause,” and 66/184 (35.9%) chose osteoporosis (Figure 2.8).

The second knowledge question asked for the percentage of women with the FMR1 premutation experience FXPOI. Results showed that 0/184 (0%) said 1%, 50/184 (27.2%) said 20%, 47/184 (25.5%) said 40%, 27/184 (14.7%) said 60%, 9/184 (4.9%) said 80% and 1/184 (0.5%) said that there was a 100% chance of developing FXPOI if an individual has the FMR1 premutation (Figure 2.9A). Of these individuals, 50/184 (27.2%) did not answer the question. When asked if the individuals themselves have experienced FXPOI, 57/184 (31%) said yes, 61/184 (33.2%) said no, 22/184 (12%) said that they were unsure, and 44/184 (23.9%) chose not to answer the question (Figure 2.9D). Of those that were unsure, most stated that they had no formal diagnosis of FXPOI but needed to see a physician because they potentially were experiencing symptoms. When asked if anyone in the family had FXPOI, 51/184 (27.7%) said yes, 45/184 (24.5%) said no, 45/184 (24.5%) said that they were unsure, and 43/184 (23.4%) chose not to answer the question. Of those that were unsure, reasons cited were that they did not know their family history or have contact with relatives who may be experiencing this. Additionally, some stated that they were unsure because it had not come up in conversation with relatives and that there were some cases of infertility in the family but the cause was unknown.

We surveyed participants about where/from whom and when they first learned about FXPOI being associated with the FMR1 premutation. Results are summarized in Figure 2.10. Regarding where or from whom they received results, 5/184 (2.7%) said “face to face support group,” 4/184 (2.2%) listed “books,” 17/184 (9.2%) listed “conversations with other [individuals with] a fragile X premutation,” 44/184 (23.9%) stated
“conversations with other healthcare providers,” 32/184 (17.4%) individuals listed “internet websites,” such as the National Fragile X Foundation website, 5/184 (2.7%) listed “online Facebook group,” and 6/184 (3.3%) listed “informational pamphlet.” In addition, 7/184 (3.8%) stated “this has never been discussed with me,” 2/184 (1.1%) individuals listed “do not know/recall,” and 52/184 (28.3%) chose not to answer the question. Of the individuals with the FMR1 premutation, 10/184 (5.4%) listed “other,” which included five individuals who first read it on published scholarly articles/research meetings.

Regarding when they first learned about FXPOI being associated with the FMR1 premutation, 35/184 (19%) said “at the time I was found to be a premutation carrier,” 7/184 (3.8%) said “at the time of my POI/POF diagnosis,” 6/184 (3.3%) stated “at the time of a family member’s POI/POF diagnosis,” 33/184 (17.9%) said “at the time of my child’s FXS diagnosis,” 8/184 (4.3%) listed “at the time of another family members’ FXS diagnosis,” and 29/184 (15.8%) listed “at another time” (Figure 2.11). Additionally, 14/184 (7.6%) individuals listed “this has never been discussed with me and 52/184 (28.3%) chose not to answer the question. Of those that listed “at another time,” responses included learned at a FXS conference or when doing research on one’s own, and one individual stated that she learned of FXPOI in graduate school.

We also asked participants when they feel like the best time is for the healthcare provider to discuss the risks of FXPOI to an individual with the FMR1 premutation. Of the individuals with the FMR1 premutation, 94/184 (51%) of individuals stated that they would prefer this information at the time of diagnosis, while 5/184 (2.7%) said that they would prefer to receive this information a few months after diagnosis or at a follow-up
appointment. Some individuals gave specifics regarding when they would want to receive this information with 5/184 (2.7%) individuals saying that they would want to receive this information at the time of family planning, 1/184 (0.5%) individual stated that it should be mentioned at a yearly physical, and 3/184 (1.6%) mentioned that it should be revealed during puberty. Additionally, 7/184 (3.8%) either did not know or did not properly answer the question. 69/184 (37.5%) of individuals left this question blank (Figure 2.12).

We sought to see which resources are most helpful for learning about FXPOI in relation to having the FMR1 premutation. Individuals could choose up to three resources which they found most helpful and results can be found in Figure 2.13. Of these participants, 24/184 (13%) listed “books”, 14/184 (7.6%) said “face to face support group,” 71/184 (38.6%) said “conversations with other carriers of a fragile X premutation,” 77/184 (41.8%) said “conversations with healthcare providers,” 75/184 (40.8%) listed “internet websites,” 57/184 (31%) listed “online Facebook group,” 28/184 (15.2%) listed “informational pamphlet,” and 6/184 (3.3%) listed “other.”

Fragile X-Associated Tremor/Ataxia Syndrome (FXTAS)

We asked two knowledge questions about FXTAS which can affect individuals with the FMR1 premutation. The first question asked the participants to choose all features associated with FXTAS. Results showed that 66/184 (35.9%) chose “mood swings,” 86/184 (46.7%) chose “dementia,” 112/184 (60.9%) chose “intention tremor,” 114/184 (62%) chose “difficulty with balance,” and 101/184 (54.9%) chose “difficulty walking,” and 81/184 (44%) chose “numbness in extremities” (Figure 2.8B).

The second knowledge question asked what percentage of men with the FMR1 premutation experience FXTAS. Results showed that 3/184 (1.6%) said 1%, 68/184 (37%)
said 25%, 33/184 (17.9%) said 50%, 22/184 (12%) said 75%, and 0/184 (0%) said that there was a 100% chance of developing FXTAS if an individual has the *FMR1* premutation (Figure 2.9B). Of these participants, 58/184 (31.5%) did not answer the question. When asked if the individuals themselves have experienced FXTAS, 6/184 (3.3%) said yes, 120/184 (65.2%) said no, 3/184 (1.6%) said that they were unsure, and 55/184 (31.5%) chose not to answer the question (Figure 2.9D). When asked if anyone in the family had FXTAS, 23/184 (12.5%) said yes, 83/184 (45.1%) said no, 21/184 (11.4%) said that they were unsure, and 57/184 (31%) chose not to answer the question. Of those that were unsure, reasons cited were that they did not know their family history or have contact with relatives who may be experiencing this. Additionally, some stated that there was no formal diagnosis but there were symptoms present in one or more of his or her family members.

We surveyed participants about where/from whom and when they first learned about FXTAS being associated with the *FMR1* premutation. Results are summarized in Figure 2.10. Regarding where or from whom they received results, 1/184 (0.5%) said “face to face support group,” 4/184 (2.2%) listed “books,” 6/184 (3.3%) listed “conversations with other [individuals with] a fragile X premutation,” 35/184 (19%) stated “conversations with other healthcare providers,” 34/184 (18.5%) individuals listed “internet websites,” such as the National Fragile X Foundation website, 3/184 (1.6%) listed “online Facebook group,” and 8/184 (4.3%) listed “informational pamphlet.” 7/184 (3.8%) stated “this has never been discussed with me,” 7/184 (3.8%) individuals listed “do not know/recall,” and 61/184 (33.2%) chose not to answer the question. Of these participants, 18/184 (9.8%) listed “other,” of which half (9/18) first read it on published scholarly articles/research meetings.
Regarding when they first learned about FXTAS being associated with the \textit{FMRI} premutation, 29/184 (15.8\%) said “at the time I was found to be a premutation carrier,” 8/184 (4.3\%) said “at the time of my FXTAS diagnosis,” 4/184 (2.2\%) stated “at the time of a family member’s FXTAS diagnosis,” 25/184 (13.6\%) said “at the time of my child’s FXS diagnosis,” 5/184 (2.7\%) listed “at the time of another family members’ FXS diagnosis,” and 38/184 (20.7\%) listed “at another time” (Figure 2.11). Additionally, 13/184 (7.1\%) individuals listed “this has never been discussed with me and 62/184 (33.7\%) chose not to answer the question. Of those that listed “at another time,” responses included learned at a FXS conference or when doing research on one’s own. Additionally, one individual learned of FXTAS in graduate school.

We also asked participants when they feel like the best time is for the healthcare provider to discuss the risks of FXTAS to an individual with the \textit{FMRI} premutation, of which 84/184 (45.7\%) of individuals stated that they would prefer this information immediately or at the time of diagnosis, while 9/184 (4.9\%) said that they would prefer to receive this information a few months after diagnosis or at a follow-up appointment. Of these individuals with the \textit{FMRI} premutation, 3/184 (1.6\%) individuals said that they would want to receive this information if symptoms presented. In addition, 8/184 (4.3\%) either did not know or did not properly answer the question and 80/184 (43.5\%) of individuals left this question blank (Figure 2.12).

We sought to see which resources are most helpful for learning about FXTAS in relation to having the \textit{FMRI} premutation. Individuals could choose up to three resources which they found most helpful and results can be found in Figure 2.13. Results show that 21/184 (11.4\%) listed “books”, 20/184 (10.9\%) said “face to face support group,” 62/184
(33.7%) said “conversations with other carriers of a fragile X premutation,” 64/184 (34.8%) said “conversations with healthcare providers,” 75/184 (40.8%) listed “internet websites,” 34/184 (23.4%) listed “online Facebook group,” 27/184 (14.7%) listed “informational pamphlet,” and 9/184 (4.9%) listed “other.”

**Fragile X Psychiatric Manifestations**

We asked two knowledge questions about the psychiatric manifestations that are potentially being seen in individuals with the *FMR1* premutation. The first question asked the participants to choose all psychiatric features potentially associated with the *FMR1* premutation, with the knowledge that only two have been reported in the literature, depression, and anxiety (including social anxiety). Of the individuals with the *FMR1* premutation, 145/184 (78.8%) of participants listed depression and 150/184 (81.5%) listed anxiety as being associated with the *FMR1* premutation (Figure 2.8C). We also included four conditions that have not been reported in the literature to be associated with the *FMR1* premutation, as well as some conditions that have not been found to have genetic causes or genetic associations for manifestation (schizophrenia, bipolar disorder, borderline personality disorder, and conduct disorder). Results showed that 13/184 (7.1%) listed schizophrenia, 29/184 (15.8%) listed bipolar disorder, 16/184 (8.7%) individuals listed borderline personality disorder, and 18/184 (9.8%) listed conduct disorder as being associated with having the *FMR1* premutation (Figure 2.8C). In addition, 15/184 (8.2%) of individuals listed “other,” which included fibromyalgia, stress, phobias, OCD, ADHD, and effects in “social dynamics.” Only 3/184 (1.6%) of individuals stated that they did not think that there were any psychiatric manifestations associated with having the *FMR1* premutation.
The second knowledge question asked what percentage of individuals with the \textit{FMR1} premutation experience psychiatric manifestations (depression and anxiety) and 7/184 (3.8%) stated 1%, 38/184 (20.7%) said 20%, 38/184 (20.7%) said 40%, 38/184 (20.7%) said 60%, 27/184 (14.7%) said 80%, and 2/184 (1.1%) said 100% of women with the \textit{FMR1} premutation experience psychiatric manifestations, 34/184 (18.5%) of individuals chose not to answer this question (Figure 2.9C). When asked if the individuals themselves have experienced any psychiatric manifestations such as depression and/or anxiety, 120/184 (65.2%) said yes, 34/184 (18.5%) said no, 7/184 (3.8%) were unsure, and 23/184 (12.5%) chose not to answer the question (Figure 2.9D). Of those that were unsure, many individuals stated that they have symptoms of depression and/or anxiety but are unsure whether it is due to having the \textit{FMR1} premutation or because of other stressors in life (e.g. having a child with FXS and/or receiving the premutation diagnosis). One respondent said, “It is depressing having a very low functioning high behavior demands kid…” while another said, “The stress and anxiety associated with discovering that you are a carrier and the implications have also triggered anxiety and depression – I don’t think this is related to the allele, it would be distressing news to anyone.” Regarding social anxiety, one individual stated “I’ve never been diagnosed with depression or anxiety, but I do seem to have some social anxiety. I don’t know what is a ‘normal’ or ‘standard’ level of social anxiety, however, and have never seen a therapist.” When asked if anyone in the family had experienced psychiatric manifestations (i.e. depression and/or anxiety), 125/184 (67.9%) said yes, 22/184 (12%) said no, and 13/184 (7.1%) were unsure. Of those who were unsure, the main reason cited was that many family members showed symptoms but did not have a formal diagnosis of a psychiatric condition.
We surveyed participants about where/from whom and when they first learned about psychiatric manifestations potentially being associated with the *FMR1* premutation. Results are summarized in Figure 2.10. Regarding where or from whom they received results, 2/184 (1.1%) said “face to face support group,” 3/184 (1.6%) listed “books,” 18/184 (9.8%) listed “conversations with other [individuals with] a fragile X premutation,” 21/184 (11.4%) stated “conversations with other healthcare providers,” 48/184 (26.1%) individuals listed “internet websites,” such as the National Fragile X Foundation website, 16/184 (8.7%) listed “online Facebook group,” 9/184 (4.9%) listed “informational pamphlet,” 16/184 (8.7%) stated “this has never been discussed with me,” 3/184 (1.6%) individuals listed “do not know/recall,” and 32/184 (17.4%) chose not to answer the question. Additionally, 16/184 (8.7%) listed “other,” which included twelve individuals who first read it on published scholarly articles/research meetings.

Regarding when they first learned about psychiatric manifestations potentially being associated with the *FMR1* premutation, 39/184 (21.2%) said “at the time I was found to be a premutation carrier,” 3/184 (1.6%) said “at the time of my mental health diagnosis,” 1/184 (0.5%) stated “at the time of a family member’s mental health diagnosis,” 28/184 (15.2%) said “at the time of my child’s FXS diagnosis,” 14/184 (7.6%) stated “at the time of another family member’s FXS diagnosis,” 34/184 (18.5%) listed “at another time,” 32/184 (17.4%) individuals listed “this has never been discussed with me, and 33/184 (17.9%) chose not to answer the question (Figure 2.11). Of those that listed “at another time,” responses included learned at a FXS conference or when doing research on one’s own.
We also asked participants when they feel like the best time is for the healthcare provider to discuss the potential association between having the *FMR1* premutation and psychiatric manifestations to an individual with the *FMR1* premutation. Results showed that 106/184 (57.6%) of individuals stated that they would prefer this information at the time of diagnosis, while 18/184 (9.8%) said that they would prefer to receive this information a few months after diagnosis or at a follow-up appointment (Figure 2.12). Additionally, 3/184 (1.6%) participants said that individuals should be told about this if they themselves present with psychiatric symptoms, with one respondent stating the following:

I wish I had been tested as a teenager when I was struggling with depression. After learning [that psychiatric manifestations and the *FMR1* premutation] were associated, I felt like my entire life made sense, it just clicked. I always wondered why I felt so different, feeling depressed for no apparent reason. I also had trouble making friends and being in social situations with large groups.

Some participants gave specific examples of when they would want to receive this information with 1/184 (0.5%) stating that they would want to receive this information at the time of family planning, 2/184 (1.1%) stated that it should be mentioned at a yearly physical, and 7/184 (3.8%) either did not know or did not properly answer the question. Additionally, 49/184 (26.6%) of individuals left this question blank.

Our next research question sought to see which resources are most helpful for learning about the potential associations between psychiatric manifestations (i.e. depression and/or anxiety) and having the *FMR1* premutation. Individuals could choose up to three resources which they found most helpful and results can be found in Figure 2.13.
Results showed that 36/184 (19.6%) listed “books”, 24/184 (13%) said “face to face support group,” 80/184 (43.5%) said “conversations with other carriers of a fragile X premutation,” 60/184 (32.6%) said “conversations with healthcare providers,” 73/184 (39.7%) listed “internet websites,” 61/184 (33.2%) listed “online Facebook group,” and 25/184 (13.6%) listed “informational pamphlet,” and 12/184 (6.5%) listed “other.” One individual listed that he or she does not seek out information and said the following:

It’s hard to separate the life of caring for someone with FXS and its impact on your life as a carrier. In the end, nothing I go through is worse than my son’s diagnosis. Therefore, it’s hard to care enough to learn more about it. Chronic caregivers don’t put themselves first and the symptoms of being a carrier are closely aligned with the outcome of being a caregiver.

*Differences Between the Two Groups*

To determine if there was a difference in psychiatric manifestations between individuals with the *FMR1* premutation versus those with the intermediate/gray zone allele, data were analyzed using Pearson’s chi-square. Results showed that there was a significantly greater number of individuals with psychiatric manifestations in the *FMR1* intermediate/gray zone allele group than what would be expected (p<0.01). To determine if there was a difference in knowledge about FXS between the two groups, an ANOVA analysis was completed. For question one, “Who has the greatest chance of inheriting a fragile X premutation?” there were significantly more individuals with the *FMR1* premutation who answered it correctly than those with the intermediate/gray zone allele (p=0.01). However, for question two, “Who has the greatest chance of having a child with fragile X syndrome?” there was no statistically significant difference between the two
groups (p=0.08). Regarding the questions that were evaluated using a Likert scale which asked questions about both conditions that are typically seen in the general population as well as those that are seen in individuals with FXS or any of its associated phenotypes, ANOVA analyses revealed significant differences between the two groups for several of these conditions. Significantly more individuals with the premutation said that there was a higher chance for early menopause (p=0.02), social anxiety (p=0.01), depression (p=0.02), and tremor/ataxia (p=0.01) by having the \textit{FMR1} premutation. The means for these values were 57\%, 63.8\%, 60.2\%, and 51.9\%, respectively.

**DISCUSSION**

This study had three main aims: (1) to learn what information individuals with the \textit{FMR1} premutation know about FXS and its known associated phenotypes, FXPOI and FXTAS, as well as the potential for psychiatric manifestations such as depression and anxiety, (2) to learn which symptoms, if any, individuals with the \textit{FMR1} intermediate/gray zone allele experience, and (3) which resources are most helpful for learning about the abovementioned features.

**Knowledge About FXS**

Results show that most individuals, both with the \textit{FMR1} premutation as well as the intermediate/gray zone allele, underwent FXS testing due to having a child with FXS. This is interesting as expansion to a full mutation in the next generation does not occur in individuals with the \textit{FMR1} intermediate/gray zone allele and there have been no reported cases of this, revealing that these individuals may have misinterpreted the question and may actually have a different genetic carrier status than was reported. However, it is unsurprising to know that this was the same for individuals with the \textit{FMR1} premutation, as
biological mothers of children with FXS are automatically said to have the premutation and are mainly getting testing for purposes of confirmation of the diagnosis.

The fact that there is a statistically significant difference in knowledge between the two groups regarding the two knowledge questions about FXS ((1) Who has the greatest chance of inheriting a fragile X premutation, and (2) Who has the greatest chance of having a child with fragile X syndrome?) indicates that genetic counselors may need to place more emphasis on the inheritance of FXS for intermediate/gray zone allele carriers so that they too understand the risks for expansion and inheritance. Additionally, while more individuals with the \textit{FMR1} premutation answered the two knowledge questions correctly, there were still many individuals who did not, indicating that there may need to be more emphasis on the inheritance of FXS for this group as well.

Individuals in both groups overestimated his or her chances for developing symptoms related to FXS and its associated phenotypes. Additionally, the fact that individuals in the intermediate/gray zone range thought that they were at a higher risk for symptoms that are typically associated with those who have the \textit{FMR1} premutation shows that we need to make intermediate allele carriers aware that their risk for these things are not higher due to their mutation status. Also, individuals in both groups said that there was a chance of developing conditions unrelated to FXS, making it imperative that counselors address the actual associations between the allele status and potential clinical associations. However, it cannot be ruled out that individuals may have thought that there was an association between his or her mutation status and symptoms unrelated to FXS such as uterine fibroids or gastric reflux simply because of the fact that the choice was there.
Suggestions for counseling included spacing out the information over multiple clinic visits. However, it can be tricky to do this in a clinical setting, especially for those who are receiving this news prenatally while undergoing carrier screening. Typically, these patients see the genetic counselor once throughout the course of the pregnancy unless other reasons indicate long term follow-up care. These individuals have children with FXS who are followed annually in genetics clinic, but it is not known how much information these parents receive about their own implications, and where the information is received. The majority of individuals underwent testing due to having a child with FXS, and it was mentioned that parents are trying to absorb so much material about the implications for their children that it is difficult to focus on the implications for themselves.

While a large number of individuals with the \textit{FMR1} premutation said that they experienced depression and/or anxiety, this is still not an association that can be made due to the presence of the \textit{FMR1} premutation. Although there have been reports in the literature about women experiencing these symptoms even without having a child affected with FXS, the possibility that these features can present due to life stressors or even other genetic and environmental contributions cannot be ruled out. Additionally, mood disturbances such as irritability and anger as well as psychiatric conditions such as depression and anxiety are associated with FXTAS, and a large number of individuals with the \textit{FMR1} premutation stated that they were “unsure” whether they had experienced FXTAS, which further makes this a possibility to consider.

\textbf{Intermediate/Gray Zone Features}

Results show that approximately half of the participants with the \textit{FMR1} intermediate/gray zone allele report experiencing depression and/or anxiety, but whether
they were diagnosed by a licensed mental health professional was not asked. The fact that there are significantly more individuals with the \textit{FMR1} intermediate/gray zone allele who experienced psychiatric manifestations than what would be expected shows that there may be an association between having the intermediate/gray zone allele and psychiatric manifestations, but more research needs to be done that would account for other intrinsic and extrinsic factors such as family history of mental illness, previous trauma/hardships, and susceptibility to mental illness due to other genetic factors. Although the numbers are statistically significant for this study in particular, the incidence of depression and anxiety are high in the general population, with about one in four individuals experiencing some type of serious mental illness over the course of his or her lifetime (“Mental Health by the Numbers, n.d.”). Therefore, based on our results, it is likely that depression and/or anxiety is not associated with the \textit{FMR1} intermediate/gray zone allele, but it is important to be mindful of these symptoms being more prevalent when counseling these individuals. Additionally, there have been no reports to date of depression or anxiety being associated with the \textit{FMR1} intermediate/gray zone allele, and more research needs to be done into the needs of these individuals. This manifestation could be due to the anxiety of having an intermediate/gray zone allele and some of the uncertainty that comes with that, although this study did not specifically delve into that aspect.

While none of the participants with the \textit{FMR1} intermediate/gray zone allele report having experienced any Parkinsonism features, four individuals did report experiencing tremors. There may have been individuals who qualified for having experienced Parkinsonism features, but perhaps did not understand what some of the medical terms were (e.g. bradykinesia, resting tremor). Additionally, while the Parkinsonism features
have been observed in both males and females with the *FMR1* intermediate/gray zone allele, most of what has been observed has been in males (Entezari, Khaniani, Bahrami, Derakhshan, & Darvish, 2017; Hall et al., 2011; Liu, Winarni, Zhang, Tassone, & Hagerman, 2013). Also, while many of these individuals do not have a clinical diagnosis of FXTAS, they did experience some of the features associated with it such as memory loss, tremors, ataxia, and gait abnormalities. This is interesting and poses an avenue for future research.

**Helpful Resources**

Individuals with the *FMR1* premutation revealed that the most helpful resources to learn about FXS were Internet websites, conversations with healthcare providers, and conversations with other individuals with a *FMR1* premutation. Online Facebook groups came in fourth and seemed to be most helpful for learning about FXPOI and the potential psychiatric manifestations associated with the *FMR1* premutation, and that Internet websites (such as the National Fragile X Syndrome Foundation website) were most helpful for learning about FXTAS. Unsurprisingly, the most helpful resource regarding learning about the potential psychiatric manifestations associated with having the *FMR1* premutation was by having conversations with other individuals with the *FMR1* premutation. With the advent of social media, many individuals have found online Facebook groups to be helpful in not only learning information about FXS and its associated phenotypes, but also to connect with other individuals who share some of the same trials and tribulations as they do, which supports Leon Festinger’s social comparison theory. The theory recognizes that “people often rely on how they stand relative to other people to assess their opinions and potential,” and social comparison refers to “the search
for and utilization of information about other persons’ standings and opinions for the purpose of self-assessment…” (Lange, Kruglanski, & Higgins, 2012). Additionally, some of the participants who answered “conversations with other individuals with a Fragile X premutation” may have been doing so on an online Facebook group, which makes it a strong possibility that these Facebook groups could actually be even more helpful than the findings indicate. Regarding support for individuals with the *FMR1* intermediate/gray zone allele, the majority of participants stated that they would be “likely” or “very likely” to utilize a support group to connect with other individuals with the intermediate/gray zone allele, especially since online Facebook support groups specifically tailored to this population do not currently exist. The creation of one of these groups may be necessary in the future to provide a medium for these individuals to learn more about the ongoing research as well as connect with others in a similar situation to themselves.

**Study Limitations**

Although sample size provided adequate power to detect statistical differences, some limitations to the study do exist. First, the Facebook groups by which the participants were recruited contain large numbers of individuals and it is likely that only individuals who are interested in research regarding FXS were the ones who participated. Therefore, our study population may not be representative of all individuals who have the *FMR1* premutation or the *FMR1* intermediate/gray zone allele. It is also likely that there was sampling bias regarding individuals in the intermediate/gray zone range, as intermediate/gray zone allele carriers who are experiencing symptoms may be the ones to be involved in these Facebook groups. Third, since these participants were recruited
through Facebook groups with most of these groups being support groups, the findings that online support groups are one of the more beneficial resources may be skewed.

The demographic make-up of the participants was homogeneous and was not representative of the general population, primarily with regards to gender and educational level. Most individuals were female and most of them had some college education or higher. The fact that the vast majority of participants were female made it more difficult to interpret the responses to questions regarding FXTAS, as it primarily affects males. The Parkinsonism features that have been observed in individuals with the FMR1 intermediate allele have primarily been observed in males as well, so having a paucity of males in this study does not rule out the fact that there could be other individuals with the intermediate/gray zone allele that are manifesting Parkinsonism features. However, due to the large number of individuals that did not respond to the question regarding whether they were male or female, there is a possibility that many of those individuals were males. Additionally, since the majority of participants were female and females have a higher incidence of internalizing anxiety and depression than males, the findings that there was a high incidence of these psychiatric manifestations may be due to this (Eaton et al., 2012).

Another point to consider is that the knowledge base of the participants may not have been entirely reflective of the individuals’ counseling experience. For one, the participants came from a variety of different countries, and there may be variation in the counseling and information provided about FXS between countries. Also, the time points at which people learned about the inheritance of FXS as well as its associations may not reflect the information given to them at the time of diagnosis. For example, FXTAS was discovered in the early 2000s, so people who received their mutation status prior to this
would likely not have been counseled about this association. Another thing to consider is that many of the participants may have pretty good knowledge of the inheritance patterns/features now, but what about right after the diagnosis? Families of older children might have had this diagnosis for 10-20 years and have had plenty of time to do reading on their own, talk with other families, etc. and answer the survey questions based on this current knowledge.

Additionally, the majority of the questions in this study were based on self-report, which could also skew the data. Individuals who stated that they have tremors, ataxia, depression, anxiety, or any of the other features surveyed in this study may not have a formal diagnosis by a healthcare provider. Additionally, there may exist some confusion regarding the terminology of FXS and its features. Anecdotal evidence of the researchers shows that some women with the \textit{FMR1} full mutation who do not have intellectual disability consider themselves to be carriers and may have filled out the survey as such.

Lastly, it is important to point out that there were a significant number of individuals, especially in the premutation group, that did not answer all of the questions in the survey. Many of the questions were left blank and may not accurately reflect the results for the group as a whole. These included basic questions such as those asking for demographic information as well as more detailed questions regarding most helpful resources and suggestions for genetic counseling. Therefore, the results may not be entirely reflective of the participants in that group. One reason for this lack of response may have been due to the length as well as repetitiveness of the survey, as most all of these questions were asked towards the end.
**Practice Implications**

These results highlight the importance of genetic counseling for both individuals with the *FMR1* premutation as well as those that are intermediate/gray zone allele carriers. Although significantly more individuals with the *FMR1* premutation correctly answered the general knowledge questions correctly than those in the intermediate/gray zone allele range, there were still several people who do not understand the genetics of FXS. This makes it even more important for genetic counselors to take extra measures to ensure that his or her patients are understanding the inheritance, as individuals in both groups are basing reproductive decision-making on their understanding of results.

Additionally, while this research does not find a significant increase in psychiatric manifestations in individuals with the *FMR1* premutation than what would be expected, the fact that these conditions exist in this population cannot be disputed, and it is important to address these issues in clinic. Some individuals stated that they felt relieved when they learned of the potential association between having the premutation and psychiatric manifestations, as it made them not feel “weak” and allowed them to re-frame their problems to consider a potential genetic component.

Lastly, learning that Internet websites, conversations with healthcare providers and conversations with other individuals with a *FMR1* premutation are most beneficial for learning about FXS, genetic counselors and other healthcare providers can refer their patients to these resources upon diagnosis of a premutation or an *FMR1* intermediate allele. With the advent of social media, many individuals have found online Facebook groups to be helpful in not only learning information about FXS and its associated phenotypes, but also to connect with other individuals in a community of support. This is something to be
mindful of since in addition to online websites, most practices offer handouts and informational packets as resources, but distributing names of some of the online Facebook groups may be even more beneficial to our patients. Additionally, in practice, it may be beneficial for genetic counselors and other healthcare providers to ask patients what types of resources would be most helpful for his or her particular learning style, and then tailor the distribution of those resources accordingly.

**Research Recommendations**

Future research could explore the phenomenon of AGG interruptions in individuals with the *FMR1* premutation. Research has shown that the presence of one or more AGG interruptions can reduce the risk for expansion in premutation alleles (Nolin et al., 2013; Latham, Coppinger, Hadd, & Nolin, 2014; Nolin et al., 2015). Therefore, it would be interesting to see whether women underwent further testing to learn their AGG status and if having AGG interruptions affected their decisions to have more children. Along with that, it would be interesting to learn whether assistive reproductive technologies were utilized or if conception was natural.

Knowledge about FXS is continuously evolving as more research is being done. Future research could ascertain participants’ knowledge of inheritance and associated phenotypes right after diagnosis, as well as during certain time points to better control for the variable of time. Regarding research for individuals with the *FMR1* intermediate/gray zone allele, it would be beneficial to know where they are learning about the risks, if any, of having this allele. Current practice guidelines do not state that there are any risks associated with having the intermediate/gray zone allele, besides the risk for expansion in future generations (Finucane et al., 2012). However, individuals are attending conferences
and are reading the publications in the literature about the potential associations and survey responses showed that some of the individuals also learned of some potential clinical consequences from their doctors. Genetic counselors and other healthcare providers need to be able to stay on top of the research being done in order to answer questions if they arise.

This study aimed to see if individuals with the \textit{FMR1} premutation experienced any psychiatric manifestations such as depression or anxiety, as those two have been documented in the form of case reports in the literature. However, this association is not yet known and further research needs to continue be done to see whether there is, in fact, a need to counsel about the potential for these features as well. Anecdotal evidence of the researcher herself has shown that many mothers of children with FXS post on social media about their anxiety and wonder whether it is related to them having the \textit{FMR1} premutation, and the research continues to show conflicting evidence. It would be beneficial to continue to study this population to ascertain this potential association even further. It would also be helpful to screen for depression and anxiety at the time of diagnosis, as many individuals may already be experiencing these features and genetic counselors can help steer these individuals to support as needed. Additionally, future research should consider the limitations proposed by this study and seek to improve upon them, such as having a sample size more representative of the general population, as well as recruiting from a multitude of other sources.

\textit{Conclusion}

FXS is a complex genetic disorder and involves numerous facets that require proper education and follow-up. While many individuals with the \textit{FMR1} premutation are
knowledgeable about the basic inheritance of FXS as well as its associated phenotypes, it is important for genetic counselors to review this information in detail with both individuals with the \textit{FMR1} premutation as well as those with the intermediate/gray zone allele. Additionally, while there are no established associations between psychiatric manifestations and the \textit{FMR1} premutation, it is important to know that many of these individuals, as well as those with the intermediate/gray zone allele, are experiencing these symptoms and may need additional care beyond the scope of our practice. We as genetic counselors are well trained to not only delve into the psychosocial realm of these patient’s experiences, but also provide resources that are best suited to their needs. With the advent of social media, many individuals are turning to online resources for support and a sense of community, and genetic counselors can use this knowledge to steer patients to resources that would be most beneficial for them.
Table 2.1 Molecular Classification and Clinical Manifestations Based on # of CGG Repeats in *FMR1*

<table>
<thead>
<tr>
<th>Allele Classification</th>
<th># of Repeats</th>
<th>Clinical Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unaffected</td>
<td>&lt;45</td>
<td>No clinical features</td>
</tr>
<tr>
<td>Intermediate/Gray Zone</td>
<td>46-54</td>
<td>Potential Parkinsonism and neuropsychiatric features (later in life)</td>
</tr>
<tr>
<td>Premutation</td>
<td>55-200</td>
<td>Fragile X-associated Tremor/Ataxia Syndrome (FXTAS), Fragile X-associated Primary Ovarian Insufficiency (FXPOI) in females, potential for psychiatric manifestations (i.e. depression, anxiety)</td>
</tr>
<tr>
<td>Full Mutation</td>
<td>&gt;200</td>
<td>Intellectual disability, autism, facial dysmorphism, ADHD</td>
</tr>
</tbody>
</table>
Table 2.2 Demographic Information

<table>
<thead>
<tr>
<th>Categories</th>
<th>Intermediate/Gray Zone</th>
<th>Premutation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Women</td>
<td>21</td>
<td>124</td>
</tr>
<tr>
<td>Did Not Answer</td>
<td>6</td>
<td>57</td>
</tr>
<tr>
<td><strong>Country of Residence</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In the United States</td>
<td>15</td>
<td>66</td>
</tr>
<tr>
<td>Outside of the United States</td>
<td>15</td>
<td>62</td>
</tr>
<tr>
<td>Did Not Answer</td>
<td>0</td>
<td>56</td>
</tr>
<tr>
<td><strong>Education</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than High School</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Some High School</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>High School Diploma or Equivalent</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Some College, Vocational, or Trade School</td>
<td>9</td>
<td>26</td>
</tr>
<tr>
<td>College Degree</td>
<td>5</td>
<td>31</td>
</tr>
<tr>
<td>Graduate or Professional Degree</td>
<td>6</td>
<td>57</td>
</tr>
<tr>
<td>Did Not Answer</td>
<td>5</td>
<td>56</td>
</tr>
<tr>
<td><strong>Total N</strong></td>
<td>30</td>
<td>184</td>
</tr>
</tbody>
</table>
Figure 2.1 All participants were asked their reasons for being tested for the *FMR1* premutation, the year of diagnosis, as well as questions regarding when they were last had a formal discussion regarding FXS and its implications with a healthcare provider. The year 1991 is of importance as that is when the *FMR1* gene was discovered and a name was given to the condition.
**Figure 2.2** Responses for individuals with the *FMR1* premutation and for intermediate/gray zone allele carriers for two general knowledge questions regarding the inheritance of Fragile X syndrome: Who has the greatest chance of inheriting a Fragile X Premutation?” and “Who has the greatest chance of having a child with Fragile X syndrome?” The correct answers are “Daughter of a male premutation carrier” and “female premutation carrier,” respectively.
Figures 2.3 Depiction of “correct” and “incorrect” answers for the two general knowledge questions: (1) Who has the greatest chance of inheriting a fragile X premutation, and (2) Who has the greatest chance of having a child with fragile X syndrome? (A) Responses for question 1 for intermediate/gray zone allele carriers, showing that 23% of individuals with the intermediate/gray zone allele answered the question correctly. (B) Responses for question 1 for individuals with the FMR1 premutation, depicting that 51% of individuals with the FMR1 premutation answered the question correctly. (C) Responses for question 2 for intermediate/gray zone allele carriers, showing that 43% of individuals with the intermediate/gray zone allele answered the question correctly. (D) Responses for question 2 for individuals with the FMR1 premutation, depicting that 64% of individuals with the FMR1 premutation answered the question correctly.
Figure 2.4 Box and whisker plot depicting individuals’ responses regarding the likelihood (from 0%-100%) of developing conditions observed in the general population, as well as those that are or may be associated with having an *FMR1* premutation or intermediate allele.
**Figure 2.5** Effects of the *FMR1* intermediate/gray zone allele (A) and the *FMR1* premutation (B) on reproductive decision-making.
Figure 2.6 Intermediate/gray zone allele carriers’ responses regarding whether they themselves have experienced any/all of the following: depression, anxiety, memory loss, Parkinsonism features, neuropathy, gait abnormalities, ataxia, and/or tremors.
Figure 2.7 Intermediate/gray zone allele carriers’ likelihood of utilizing a support group. 24/30 (80%) of individuals with the \textit{FMR1} intermediate/gray zone allele said that they would be “likely” or “very likely” to utilize a support group if available.
Figure 2.8 Participants’ responses when asked to choose any and all features associated with either (A) FXPOI, (B) FXTAS, or (C) FX psychiatric manifestations.
Figure 2.9 Responses to knowledge questions regarding the percentage of individuals with the *FMR1* premutation who will experience (A) FXPOI, (B) FXTAS, and (C) FX psychiatric manifestations, with the correct answers being 20%, 50%, and 40%, respectively. (D) depicts whether or not these individuals themselves have experienced any of the abovementioned features.
184 individuals with the *FMR1* premutation were asked to provide information regarding which source notified them of the association between having the premutation and FXPOI, FXTAS, and the potential FX psychiatric manifestations. The overall consensus shows that individuals first learned of this information on Internet websites, conversations with other healthcare providers, and conversations with other individuals with the *FMR1* premutation.
Figure 2.11 184 individuals with the *FMR1* premutation were asked to provide information regarding when they were first notified of the association between having the premutation and FXPOI, FXTAS, and the potential FX psychiatric manifestations. The overall consensus shows that individuals first learned of this information at the time of his or her own diagnosis of having the *FMR1* premutation, at the time of his or her child’s diagnosis of FXS, or at another time (which, of those who elaborated, primarily involved attendance at regional and national FXS conferences).
Figure 2.12 184 individuals with the $FMR1$ premutation were asked what time is most helpful for learning about FXS and its associated phenotypes, and ~100 individuals (>50%) indicated that they would like to learn about these other conditions at the time of diagnosis, while only a few wanted to learn this information at a follow-up appointment. Some of those who stated “other” mentioned learning about them when symptoms began to present or that they would like to learn this information over a period of time, with multiple follow-up appointments.
Figure 2.13 Individuals with the *FMR1* premutation were instructed to choose up to three (3) resources which they found most helpful for learning about FXPOI, FXTAS, and the psychiatric manifestations that may potentially be associated with having the *FMR1* premutation. Consensus shows that internet websites (such as the National FXS Foundation website, as well as other reputable sources), conversations with other healthcare providers, and conversations with other individuals with the *FMR1* premutation are most helpful for learning more information about FXS and its associated phenotypes.
REFERENCES


Hagerman, R. J., & Hagerman, P. J. (2002). *Fragile X Syndrome: Diagnosis, Treatment, and Research*. Taylor & Francis US.


APPENDIX A

ADVERTISEMENT FOR SOCIAL MEDIA

The following advertisement was posted on various Facebook groups.

Hello,

My name is Zahra Girnary and I am a genetic counseling student at the University of South Carolina. For my thesis project, I want to study individuals who lives are affected by fragile X syndrome, but do not have fragile X syndrome themselves.

If you are a carrier of either a fragile X premutation allele or an intermediate/gray zone allele, please consider completing my questionnaire to study patients’ perceptions of clinical risk. I also want to learn which resources, if any, are most helpful to patients so that we can improve upon them and help the community at large.

This questionnaire should take about 20 minutes to complete.
https://www.surveymonkey.com/r/USC_FragileX

Thank you so much!

Zahra
APPENDIX B

FMRI PREMUTATION SURVEY

The following survey was distributed on various online Facebook groups and targeted individuals with the FMRI premutation.

Hello,

Thank you for your interest in this study. You are being asked to participate in this research study because you are either a fragile X premutation allele carrier or a fragile X intermediate/gray zone allele carrier. Your participation in this study is completely voluntary and all responses are anonymous and will be kept confidential. Informed consent will be implied upon completion of the questionnaire, but you may stop your participation at any time or choose not to answer specific questions without any consequence, as some questions may touch on sensitive topics.

The online questionnaire should take approximately 20 minutes to complete and there will be no course credit or monetary compensation given for your participation. However, your time is greatly appreciated and we hope the results will further research in the fragile X community.

If you have any questions, please do not hesitate to contact me by email at zahra.girnary@uscmed.sc.edu. Additionally, if you would like to learn the results of the study after its completion, you may provide your email at the end of the survey.

Sincerely,

Zahra Girnary
Genetic Counseling Student
University of South Carolina – Columbia

1) By clicking “Yes” I have read the above and choose to participate in this study.
   Yes

2) I am a fragile X
Intermediate/Gray Zone Allele Carrier (41-54 CGG repeats)
Premutation Allele Carrier (Between 55-200 CGG repeats)

3) When you received your premutation result, what information did you receive from your healthcare provider about the implications for your own health?

4) Has the size of your premutation affected your reproductive decisions? If so, how and why?

5) If you have any family members diagnosed with fragile X syndrome, please list their relationship to you and approximate year of diagnosis in the space provided.

6) When was the last time you had a formal discussion with your healthcare provider regarding fragile X and its implications?
   - Never
   - Unsure
   - Before 1990
   - Between 1991 – 2017

7) (If they select “Between 1991-2017”) Please select the year you had a formal discussion regarding fragile X syndrome and its implications with a healthcare provider.
   1991 2004 2017

8) When were you found to be a premutation carrier?
   - Unsure
   - Before 1990
   - Between 1991 – 2017

9) (If they select “Between 1991-2017”) Please select the year you were found to be a premutation carrier.
   1991 2004 2017

10) What was the indication for you being tested for a premutation?
    - I have a child/children with fragile X syndrome
    - I have a family history of fragile X syndrome
    - I have a family member known to be a premutation carrier
    - Prenatal carrier screening
    - Other (please specify)
    - I have not been tested (please explain)
11) (If they selected “Other” or “I have not been tested”) You selected “Other” or “I have not been tested”. Please explain your answer in the space provided below.

12) Did you have genetic counseling at the time you were found to be a premutation carrier?
   Yes
   No
   Unsure

The following questions are general knowledge questions about the inheritance of Fragile X syndrome.

13) Who has the greatest chance of inheriting a fragile X premutation?
   Daughter of a male premutation carrier
   Daughter of a female premutation carrier
   Son of a male premutation carrier
   Son of a female premutation carrier
   All have the same chance
   Unsure

14) Who has the greatest chance of having a child with fragile X syndrome?
   Female premutation carrier
   Male premutation carrier
   Both have the same chance of having a child with fragile X
   Unsure

The following questions include conditions that are commonly observed in the general population. For each condition, please indicate how likely you believe the condition is to be observed in individuals who carry a fragile X premutation allele.

1) What is the likelihood of a fragile X premutation allele causing heart disease in a premutation carrier?
   0 → 100%

2) What is the likelihood of a fragile X premutation allele causing kidney problems in a premutation carrier?
   0 → 100%
3) What is the likelihood of a fragile X premutation allele causing uterine fibroids in a premutation carrier?
   0 → 100%

4) What is the likelihood of a fragile X premutation allele causing early menopause in a premutation carrier?
   0 → 100%

5) What is the likelihood of a fragile X premutation allele causing diabetes in a premutation carrier?
   0 → 100%

6) What is the likelihood of a fragile X premutation allele causing social anxiety in a premutation carrier?
   0 → 100%

7) What is the likelihood of a fragile X premutation allele causing depression in a premutation carrier?
   0 → 100%

8) What is the likelihood of a fragile X premutation allele causing tremor/ataxia in a premutation carrier?
   0 → 100%

9) What is the likelihood of a fragile X premutation allele causing gastric reflux in a premutation carrier?
   0 → 100%

10) What is the likelihood of a fragile X premutation allele causing glaucoma in a premutation carrier?
    0 → 100%

The following questions are related to fragile-X-associated psychiatric manifestations.

1) Psychiatric manifestations associated with being a carrier of the fragile X premutation allele can involve which of the following (select all that apply):
   - Depression
   - Anxiety
   - Schizophrenia
   - Bipolar disorder

90
Borderline personality disorder
Conduct disorder
Other ______________________

2) Approximately ________ percent of women carrying a fragile X premutation experience psychiatric manifestations.
   1%
   20%
   40%
   60%
   80%
   100%

3) Have you experienced psychiatric manifestations (i.e. depression and/or anxiety)?
   Yes
   No
   Unsure

4) (If they selected “Unsure”) You selected “Unsure”, please explain in the space provided below.

5) Has anyone in your family experienced psychiatric manifestations (i.e. depression and/or anxiety)?
   Yes
   No
   Unsure

6) (If they selected “Unsure”) You selected “Unsure”, please explain in the space provided below.

7) WHERE or FROM WHOM did you FIRST learn that psychiatric manifestations were associated with the fragile X premutation?
   This has never been discussed with me
   Do not know/recall
   Face to face support group
   Book(s)
   Conversations with other carriers of a fragile X premutation
   Internet website(s) (please specify what website)
   Online Facebook group (please specify which one)
Informational pamphlet (please specify from whom you received the pamphlet below)
Conversations with healthcare providers (please specify what type)
Other (please specify)
For responses asking for additional information, please use the space provided below.

8) WHEN did you FIRST learn that psychiatric manifestations were associated with the fragile X premutation?
   This has never been discussed with me
   At the time I was found to be a premutation carrier
   At the time of my mental health diagnosis
   At the time of a family member’s mental health diagnosis
   At the time of my child’s fragile X syndrome diagnosis
   At the time of another family member’s fragile X syndrome diagnosis
   At another time (please explain at what time below)
   Please explain when you found out that psychiatric manifestations were associated with the fragile X premutation.

9) In the space provided, please describe when you believe is the best time for the healthcare provider to first discuss the association between fragile X syndrome and psychiatric manifestations with a carrier of a premutation.

10) Choose THREE (3) resources you find the most helpful in learning about psychiatric manifestations associated with being a carrier of the fragile X premutation.
    Book(s)
    Face to face support group
    Conversations with other carriers of a fragile X premutation
    Conversations with healthcare providers (please specify what type)
    Internet website(s) (please specify what website)
    Online Facebook group (please specify which group)
    Informational pamphlet (please specify from whom you received the pamphlet below)
    Other (please specify)
    For responses asking for additional information, please use the space provided below.
11) In the space provided below please list any suggestions for genetic counseling for psychiatric manifestations associated with being a carrier of the fragile X premutation.

The following questions are related to fragile-X-associated primary ovarian insufficiency (FXPOI).

1) Fragile X-associated primary ovarian insufficiency (FXPOI), previously known as premature ovarian failure (POF), can involve which of the following (select all that apply):
   - Menstrual cycle irregularities
   - Hormone fluctuations
   - Decreased fertility
   - Early onset menopause
   - Osteoporosis

2) Approximately ________ percent of women carrying a fragile X premutation experience FXPOI/POF.
   - 1%
   - 20%
   - 40%
   - 60%
   - 80%
   - 100%

3) Have you experienced fragile FXPOI/POF?
   - Yes
   - No
   - Unsure

4) (If they selected “Unsure”) You selected “Unsure”. Please explain in the space provided below

5) Has anyone in your family experienced FXPOI/POF?
   - Yes
   - No
   - Unsure

6) (If they selected “Unsure”) You selected “Unsure”. Please explain in the space provided below
7) WHERE or FROM WHOM did you first learn that POI/POF was associated with the fragile X premutation?
   - This has never been discussed with me
   - Do not know/recall
   - Face to face support group
   - Book(s)
   - Conversations with other carriers of a fragile X premutation
   - Internet website(s) (please specify what website)
   - Online Facebook groups (please specify which one)
   - Informational pamphlet (please specify from whom you received the pamphlet below)
   - Conversations with healthcare providers (please specify what type)
   - Other (please specify)

   For responses asking for additional information, please use the space provided below.

8) WHEN did you first learn that POI/POF was associated with the fragile X premutation?
   - This has never been discussed with me
   - At the time I was found to be a premutation carrier
   - At the time of my POI/POF diagnosis
   - At the time of a family member’s POI/POF diagnosis
   - At the time of my child’s fragile X syndrome diagnosis
   - At the time of another family member’s fragile X syndrome diagnosis
   - At another time (please explain at what time below)

   If you selected “At another time”, please explain in the space provided below.

9) In the space provided below, please describe when you believe is the best time for the healthcare provider to first discuss the association between fragile X syndrome and FXPOI/POF with a carrier of a premutation.

10) Choose THREE (3) resources you find the most helpful in learning about psychiatric manifestations associated with being a carrier of the fragile X premutation.
   - Book(s)
   - Face to face support group
   - Conversations with other carriers of a fragile X premutation
   - Conversations with healthcare providers (please specify what type)
   - Internet website(s) (please specify what website)
   - Online Facebook group (please specify which group)
Informational pamphlet (please specify from whom you received the pamphlet below)
Other (please specify)
For responses asking for additional information, please use the space provided below.

11) In the space provided below, please list any suggestions for genetic counseling for FXPOI/POF.

The following questions are related to fragile-X-associated tremor/ataxia syndrome (FXTAS).

1) Fragile X-associated tremor/ataxia syndrome (FXTAS) can involve which of the following (select all that apply):
   Mood swings
   Dementia
   Intention tremor
   Difficulty with balance
   Difficulty walking
   Numbness in extremities

2) Approximately ________ percent of men carrying a fragile X premutation experience FXTAS.
   1%
   25%
   50%
   75%
   100%

3) Have you been diagnosed with FXTAS?
   Yes
   No
   Unsure

4) (If they selected “Unsure”) You selected “Unsure”. Please explain in the space provided below

5) Has anyone in your family been diagnosed with FXTAS?
   Yes
   No
   Unsure
6) (If they selected “Unsure”) You selected “Unsure”. Please explain in the space provided below

7) WHERE or FROM WHOM did you first learn that tremor/ataxia was associated with the Fragile X premutation?
   - This has never been discussed with me
   - Do not know/recall
   - Face to face support group
   - Book(s)
   - Conversations with other carriers of a fragile X premutation
   - Internet website(s) (please specify what website)
   - Online Facebook group (please specify which one)
   - Informational pamphlet (please specify from whom you received the pamphlet below)
   - Conversations with healthcare providers (please specify what type)
   - Other (please specify)

For responses asking for additional information, please use the space provided below.

8) WHEN did you first learn that tremor/ataxia was associated with the fragile X premutation?
   - This has never been discussed with me
   - At the time I was found to be a premutation carrier
   - At the time of my POI/POF diagnosis
   - At the time of a family member’s POI/POF diagnosis
   - At the time of my child’s fragile X syndrome diagnosis
   - At the time of another family member’s fragile X syndrome diagnosis
   - At another time (please explain at what time below)

Please explain when you found out that FXTAS was associated with the fragile X premutation.

9) In the space provided below, please describe when you believe is the best time for the healthcare provider to first discuss the association between fragile X syndrome and FXTAS with a carrier of a premutation.

10) Choose THREE (3) resources you find the most helpful in learning about psychiatric manifestations associated with being a carrier of the fragile X premutation.
    - Book(s)
    - Face to face support group
    - Conversations with other carriers of a fragile X premutation
Conversations with healthcare providers (please specify what type)
Internet website(s) (please specify what website)
Online Facebook group (please specify which group)
Informational pamphlet (please specify from whom you received the pamphlet below)
Other (please specify)
For responses asking for additional information, please use the space provided.

11) In the space provided below, please list any suggestions for genetic counseling for FXTAS.

Demographic Questions

1) I am a biological __________
   Male
   Female

2) I am _____ years old.

3) I live______________.
   In the United States
   Outside the United States

4) (If they choose “In the United States”) Which state do you live in?

5) (If they choose “Outside the United States”) Which country do you live in?

6) What is your highest level of education?
   Less than high school
   Some high school
   High school diploma or equivalent
   Some college, vocational or trade school
   College degree
   Graduate or professional degree (please specify)

7) (If they selected “Graduate or Professional Degree”) What is your graduate or professional degree (i.e., MS, PhD, MD, JD, etc.)?

8) What is your occupation?
9) In the space provided below, please indicate the website or listserv where you learned about this survey

10) If you’d like to receive the results of this study, please provide your email address in the space provided below.
APPENDIX C

FMRI INTERMEDIATE/GRAY ZONE ALLELE SURVEY

The following survey was distributed on various online Facebook groups and targeted individuals with the FMRI intermediate/gray zone allele.

Hello,

Thank you for your interest in this study. You are being asked to participate in this research study because you are either a fragile X premutation allele carrier or a fragile X intermediate/gray zone allele carrier. Your participation in this study is completely voluntary and all responses are anonymous and will be kept confidential. Informed consent will be implied upon completion of the questionnaire, but you may stop your participation at any time or choose not to answer specific questions without any consequence, as some questions may touch on sensitive topics.

The online questionnaire should take approximately 20 minutes to complete and there will be no course credit or monetary compensation given for your participation. However, your time is greatly appreciated and we hope the results will further research in the fragile X community.

If you have any questions, please do not hesitate to contact me by email at zahra.girnary@uscmed.sc.edu. Additionally, if you would like to learn the results of the study after its completion, you may provide your email at the end of the survey.

Sincerely,

Zahra Girnary
Genetic Counseling Student
University of South Carolina – Columbia

1) By clicking “Yes” I have read the above and choose to participate in this study.
   Yes

2) I am a fragile X
Intermediate/Gray Zone Allele Carrier (41-54 CGG Units)
Premutation Allele Carrier (55-200 CGG Units)

3) When you received your gray zone/intermediate allele result, what information did you receive from your healthcare provider about the implications for your own health?

4) If you have any family members diagnosed with fragile X syndrome, please list their relationship to you and approximate year of diagnosis in the space provided.

5) When was the last time you had a formal discussion with your healthcare provider regarding fragile X syndrome and its implications?
   When I was found to be a gray zone carrier
   Never
   Unsure
   Before 1990
   Between 1991 – 2017

6) (If they select “Between 1991-2017”) Please select the year you had a formal discussion regarding fragile X syndrome and its implications with a healthcare provider.
   1991
   2004
   2017

7) When were you found to be an intermediate/gray zone allele carrier?
   Unsure
   Before 1990
   Between 1991 – 2017

8) (If they select “Between 1991-2017”) Please select the year you were found to be an intermediate/gray zone allele carrier.
   1991
   2004
   2017

9) What was the indication for you being tested for fragile X?
   I have a child/children with fragile X syndrome
   I have a family history of fragile X syndrome
   I have a family member known to be a premutation carrier
   Prenatal carrier screening
   Other (please specify)
   I have not been tested (please explain)
   Unsure
10) (If they selected “Other” or “I have not been tested”) You selected “Other” or “I have not been tested”. Please explain your answer in the space provided below.

11) Did you have genetic counseling at the time you were found to be a gray zone carrier?
   - Yes
   - No
   - Unsure

12) Have you or anyone in your family been diagnosed with Parkinson’s Disease?

13) Have you experienced any of the following features? (Select all that apply)
   - Tremor
   - Ataxia
   - Gait abnormalities
   - Neuropathy
   - Parkinsonism (Defined as having 2 of the following: bradykinesia, resting tremor, rigidity, asymmetric onset)
   - Memory loss
   - Anxiety
   - Depression

14) Has your gray zone result affected your reproductive decisions? If so, how and why?

15) There exist many online support groups for individuals with fragile X syndrome as well as those who are premutation allele carriers. How likely would you be to access a support group for gray zone/intermediate allele carriers?
   - Very unlikely
   - Unsure
   - Likely
   - Very likely

16) (If they answered “Likely” or “Very Likely”) You answered “Likely” or “Very Likely” in the previous question. “How do you think a support group would benefit you?”

**The following questions are general knowledge questions about the inheritance of Fragile X syndrome.**

17) Who has the greatest chance of inheriting a fragile X premutation?
   - Daughter of a male premutation carrier
   - Daughter of a female premutation carrier
   - Son of a male premutation carrier
Son of a female premutation carrier
All have the same chance
Unsure

18) Who has the greatest chance of having a child with fragile X syndrome?
Female premutation carrier
Male premutation carrier
Both have the same chance of having a child with fragile X
Unsure

The following questions include conditions that are commonly observed in the general population. For each condition, please indicate how likely you believe the condition is to be observed in individuals who carry a Fragile X intermediate allele. 
*An intermediate allele is defined as having between 41 and 54 CGG units.*

1) What is the likelihood of a fragile X intermediate allele causing heart disease in an intermediate allele carrier?
   $0 \rightarrow 100\%$

2) What is the likelihood of a fragile X intermediate allele causing kidney problems in an intermediate allele carrier?
   $0 \rightarrow 100\%$

3) What is the likelihood of a fragile X intermediate allele causing uterine fibroids in an intermediate allele carrier?
   $0 \rightarrow 100\%$

4) What is the likelihood of a fragile X intermediate allele causing early menopause in an intermediate allele carrier?
   $0 \rightarrow 100\%$

5) What is the likelihood of a fragile X intermediate allele causing diabetes in an intermediate allele carrier?
   $0 \rightarrow 100\%$

6) What is the likelihood of a fragile X intermediate allele causing social anxiety in an intermediate allele carrier?
   $0 \rightarrow 100\%$

7) What is the likelihood of a fragile X intermediate allele causing depression in an intermediate allele carrier?
8) What is the likelihood of a fragile X intermediate allele causing tremor/ataxia in an intermediate allele carrier?
   0 → 100%

9) What is the likelihood of a fragile X intermediate allele causing gastric reflux in an intermediate allele carrier?
   0 → 100%

10) What is the likelihood of a fragile X intermediate allele causing glaucoma in an intermediate allele carrier?
    0 → 100%

Demographic Questions

19) I am a biological _________
   Male
   Female

20) I am _____ years old.

21) I live_________________.
    In the United States
    Outside the United States

22) (If they choose “In the United States”) Which state do you live in?

23) (If they choose “Outside the United States”) Which country do you live in?

24) What is your highest level of education?
   Less than high school
   Some high school
   High school diploma or equivalent
   Some college, vocational or trade school
   College degree
   Graduate or professional degree (please specify)

25) (If they selected “Graduate or Professional Degree”) What is your graduate or professional degree (i.e., MS, PhD, MD, JD, etc.)?
26) What is your occupation?

27) In the space provided below, please indicate the website or listserv where you learned about this survey.

28) If you’d like to receive the results of this study, please provide your email address in the space provided below.
APPENDIX D

SURVEY DEBRIEFING FORM

For participants in the United States:
Thank you for agreeing to participate in this research. You may find the nature of some of the questions to be personal and/or sensitive. If you feel that you may benefit from psychological services, there are options available.

One option is to seek either treatment or an appropriate referral from either your primary care physician or personal psychologist. You may also contact the national crisis hotline at (775) 784-8090 or the National Suicide Prevention Hotline at 1 (800) 273-8255.

If you ever feel as though you may be a danger to yourself or others, it is important that you seek help immediately. If your emergency takes place after business hours, please call 911 or go to the emergency room of your nearest hospital.

For international participants:
Thank you for agreeing to participate in this research. You may find the nature of some of the questions to be personal and/or sensitive. If you feel that you may benefit from psychological services, there are options available.

One option is to seek either treatment or an appropriate referral from either your primary care physician or personal psychologist. You may also wish to visit the International Association for Suicide Prevention (IASP) at http://www.iasp.info/resources/Crisis_Centres/ and/or BetterHelp at https://www.betterhelp.com/gethelpnow/ to find a crisis center near you.

If you ever feel as though you may be a danger to yourself or others, it is important that you seek help immediately. If your emergency takes place after business hours, please call your local emergency number or go to the emergency room of your nearest hospital.