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Tremor and Other Hyperkinetic Movements

Viewpoints

In the Gray Zone in the Fragile X Gene: What are the Key Unanswered Clinical and Biological Questions?

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Abstract

Smaller expansions (41–54 CGG repeats) in the *fragile X mental retardation 1* (*FMR1*) gene are termed "gray zone" alleles. Only recently has interest in these expansions increased due to reporting of phenotypes unique to gray zone carriers or similar to those seen in individuals with larger expansions. As minimal research has focused on gray zone expansions, this paper asks several questions related to this topic. These include the following: What is the definition of the gray zone? Is there a risk of developing neurological signs in these carriers? Are there secondary gene effects that impact gray zone alleles or a biologic advantage to carrying these repeats? How do we counsel patients with gray zone expansions? The answers to these questions will help to determine the significance of these expansions and provide needed information to the research community and clinicians.

Keywords: Fragile X mental retardation 1 Gene, Fragile X-associated tremor/ataxia syndrome, Movement disorders

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Introduction

Trinucleotide repeat expansions (CGG) in the 5′ untranslated region of the *fragile X mental retardation 1* (*FMR1*) gene are associated with neurological signs in adults. Fragile X syndrome results from a full expansion (>200 CGG repeats) in the gene and is the most common inherited form of intellectual disability in boys. It is characterized by decreased cognitive function, developmental anomalies, learning disabilities, and emotional problems. Fragile X syndrome typically presents in childhood and is not typically a progressive disorder with tremor and ataxia. Repeat expansions in *FMR1* of 55–200 CGG repeats, or the premutation range, can manifest as the fragile X-associated tremor/ataxia syndrome (FXTAS) or premature ovarian insufficiency.[1]

FXTAS is a progressive neurological disorder with intention tremor, cerebellar ataxia, and parkinsonism that was described initially in 2001 by our research group.[2] FXTAS was discovered by a developmental pediatrician who noticed that the grandparents of boys with fragile X syndrome had tremor and balance problems. The classic phenotype is typically seen in male premutation carriers over the age of 50, but it has now been reported in female premutation carriers.[2–5] FXTAS-like phenotypes have also been reported in full mutation carriers.[6–9] Other features of FXTAS include peripheral neuropathy, lower limb proximal muscle weakness, autonomic dysfunction, progressive cognitive decline, and behavioral problems.[10,11]

Smaller expansions in *FMR1* have been termed "gray zone" or intermediate alleles based on the lower likelihood of the CGG repeat increasing and causing fragile X syndrome in later generations. Gray zone alleles have been reported to expand over two generations to a full mutation, but typically it takes at least three generations.[12–14]

Due to the location of the *FMR1* gene on the X chromosome, the prevalence rates of repeat expansions are reported by sex. Mutations leading to fragile X syndrome occur in one out of 4,000 males and one out of 8,000 females.[15–18] The premutation occurs in one out of 209 females and one out of 430 males.[19,20] It is hypothesized that the prevalence rates may be more proportional between men and women than previously reported,[21] but this has not been confirmed by studies.

The rate of *FMR1* gray zone expansions in the general population is variable, but large population studies report rates of 0.8% to 3.0% for repeat sizes between 41 and 54.[19,20,22] However, many of the

Table 1. Prevalence of Gray Zone Alleles in Population Studies

Author	Defined Gray Zone	Age	Gender	No. of Patients	Location	Gray Zone Rate (%)
Rousseau et al.[56]	35–54	Not reported	Women	10,624	Canada	0.4
Dawson et al.[57]	40–59	Newborns	Men and women	2,000	Spain	2.7
Zhong et al.[58]	41–54	Not reported	Men	56	USA	1.7
Spence et al.[59]	40–59	(Pregnant women of advanced maternal age)	Women	745	USA	2.2
Drasinover et al.[52]	50–55	Not reported	Women	10,587	Israel	0.5
Dombrowski et al.[60]	40–54	Not reported	Men	10,572	Canada	0.3
Penagarikano et al.[22]	41–54	Not reported	Men	158	Spain	3.2
Cronister et al.[61]	45–54	Not reported	Women	29,103	USA	0.7
Tzeng et al.[62]	45–54	Newborns	Boys	10,046	Taiwan	0.7
Metcalfe et al.[63]	45–54	>18 years	Women	338	Australia	0.7
Levesque et al.[64]	45–54	Mother-newborn pairs	Women, boys, and girls	24,449	Canada	1.2
Fernandez-Carvajal et al.[14]	45–54	Newborns	Boys and girls	5,267	Spain	3.8
Otsuka et al.[65]	40–50	Not reported	Men and women	946	Japan	0.6
Tassone et al.[19]	45–54	Newborns	Boys and girls	14,207	USA	1.2
Seltzer et al.[66]	45–54	67–68 years	Men and women	6,747	USA	2.6

epidemiological studies done have variable definitions of the gray zone, ranging between 34 and 60 repeats making comparison across studies difficult (Table 1).

Given the recent increasing interest in smaller expansions in the *FMR1* gene, this paper will summarize some of the key issues that need to be addressed in this area. For the purposes of this paper, "gray zone" terminology will be used. These issues impact not only fragile X researchers; but also clinicians, genetic counselors, and geneticists who will be seeing these individuals for clinical care.

What FMR1 *repeat size should be defined as the gray zone?*

The American College of Medical Genetics practice guidelines define the intermediate zone or gray zone alleles as 41–60 CGG repeats.[23] However, 45–54 CGG repeats have been designated the intermediate or gray zone by the laboratory practice committee of the American College of Medical Genetics.[24,25] More recent population studies have used 41–54 repeats.[19] This discrepancy in the definition of the gray zone has become more important recently because several studies have now reported phenotypes associated with gray zone or intermediate allele sizes. 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. It is likely with more research, especially in regards to the molecular consequences of smaller expansions, a clearer delineation of what constitutes the gray zone or intermediate alleles will be apparent.

Is there a risk of developing neurological signs in individuals with repeats from 41–54?

Until recently, *FMR1* gray zone expansions (41–54 CGG repeats) were not thought to be associated with disease or neurological signs. In

2000, a 5-year survey of special education boys in the United Kingdom showed an excess of gray zone expansions.[26] However, this result has not been replicated in other populations.[27] In 2006, it was recognized that premature ovarian insufficiency, which is associated with premutation expansions, is also present in gray zone expansion carriers.[28,29]

More recently, our group found 5.5% of parkinsonism patients (n=273) overall in movement disorder clinics were *FMR1* gray zone carriers and that 12% of the female parkinsonism patients (11/98) had gray zone alleles.[30] Clinical characteristics of the gray zone carriers included classical features of Parkinson disease (PD), with most patients having asymmetric rest tremor, bradykinesia, and rigidity. In addition, gray zone carriers were dopamine responsive and some had evidence of motor fluctuations and dyskinesia from dopaminergic medications. However, some of the gray zone carriers with parkinsonism had features uncommon in idiopathic PD, to include kinetic tremor and mild gait ataxia. Although women with parkinsonism were the most highly represented among the gray zone carriers in this screening study, the rate of *FMR1* gray zone expansions was also increased in women with ataxia (n=75) compared to controls (n=115): 9% vs. 4.3% ($p<0.001$). Other clinical features included a higher rate of anxiety in the gray zone carrier women, which is a common feature seen in premutation (55–200 CGG) carrier women, with or without FXTAS.[31]

Two other groups have reported increased gray zone expansions in parkinsonism populations. In an Australian cohort, 228 males with PD or parkinsonism seen in a movement disorder clinic were screened.[32] Compared to newborn males screened at the same institution (n=576), there was an increased prevalence of gray zone expansions (8% vs. 3.4%, $p=0.012$). A second group in China screened 360 patients with parkinsonism and 295 age and sex-matched controls and found similar results, with an increased rate of *FMR1* gray zone expansions in female cases (n=147) compared to female controls (n=122; 6.8% vs. 0, $p<0.05$).[33] However, no association was seen in the Chinese men with parkinsonism. An additional group found two men with PD and marked cognitive decline with 41 and 46 CGG repeats and postulate that a possible association may exist in phenotypes on the PD spectrum.[34]

In contrast, other studies have not shown an increase in gray zone or intermediate alleles in similar populations.[35] The rates reported are variable, with 1.2–1.9% of male parkinsonism patients, 3% of Asian late-onset PD patients, and 2.3% of Italian female parkinsonism patients having gray zone alleles.[36–38] Although all of these studies reported that these rates are the same as the population rate, women have been excluded in many of the screens due to the definition of FXTAS being seen initially in male premutation carriers only. Given the differences of these study results in sex, ethnicity, and control populations, defining the true association in a community-based unbiased sample is important, but unlikely to be accomplished as parkinsonism affects such a small percentage of the population.

Population screening studies would also suggest that gray zone alleles are more highly represented in ataxia phenotypes in general, including multiple system atrophy, with rates as high as 8%.[39] However, the prevalence rates are difficult to compare across studies given the different study designs, with varying types of ataxia patients included, mixed populations, differing ages, and lack of collected controls.

A FXTAS-like phenotype has now been reported in gray zone carriers. A study of a family of *FMR1* repeat expansion carriers with broad clinical involvement describes a gray zone carrier with 52 CGG repeats with subtle tremor and balance problems, but milder than what is seen in FXTAS.[40] Imaging was not reported. In addition, three gray zone carriers who meet clinical diagnostic criteria for FXTAS have been described by our group[41] and two additional cases by a second group.[42] Like the earlier studies, which may expand the phenotype of FXTAS, better characterization of gray zone carriers with neurological signs and symptoms may suggest that these individuals also have a FXTAS-like phenotype or a neurological phenotype more similar to PD.

Do individuals with a gray zone expansion and parkinsonism have a different entity altogether from PD?

In order to investigate differences in parkinsonism in *FMR1* gray zone expansions compared to typical PD, our group conducted a study to determine if presynaptic dopamine deficits were present in the *FMR1* repeat expansion carriers. Dopamine transporter imaging with [^{123}I] β-CIT (2β-carbomethoxy-3β-(4-iodophenyl) tropane) SPECT (single photon emission computed tomography) was performed on two patients with parkinsonism and a gray zone expansion.[30,43] Both patients had normal imaging despite having parkinsonism, meeting clinical criteria for PD, and responding to dopaminergic therapy. This suggests that parkinsonism associated with *FMR1* repeat expansion may be different mechanistically than typical PD.

Are pathologic changes or neuroimaging abnormalities present in gray zone carriers with neurological signs?

Gray zone carriers of 45–54 CGG repeats can have up to 1.5-fold increase in *FMR1* mRNA, with transcript levels of *FMR1* mRNA starting to increase at just 39 CGG repeats.[44] This increase in *FMR1* mRNA is seen to a greater extent (two–fourfold) in carriers of premutation size expansions and has been postulated to account for a "toxic gain of function" mechanism leading to neurodegeneration in FXTAS.[45] Higher levels of mRNA correlate positively with degree of neuropathology[46] and peripheral neuropathy[47] in affected premutation carriers, which supports this hypothesis. It is not clear whether a similar mechanism accounts for neurological signs in gray zone carriers or whether other pathophysiology is present.

There is a paucity of neuroimaging data reported in gray zone carriers. Loesch et al.[48] reported a 71-year-old male with a PD phenotype and dementia who had T2 hyperintensity in the basis pontis. Abnormalities in the pontine fibers that are entering or exiting the cerebellum, especially in the middle cerebellar peduncle (MCP), may be evidenced in premutation carriers as a hyperintensity in the MCP and has been associated with FXTAS.[49]

Are other secondary gene effects responsible for the phenotypes seen in gray zone expansion carriers?

A study that examined patients with gray zone or low end premutation expansions (40–85 CGG repeats) showed that motor dysfunction and cognitive decline were correlated with CGG repeat size, levels of antisense *FMR1*, and *cytochrome C1* (*CYC1*) mRNA.[50] The antisense transcript has been shown to be elevated in premutation carriers and may be a secondary gene effect that increases the likelihood of manifestation of neurological signs in *FMR1* expansion carriers. The authors showed that the relationship between CGG repeat size, antisense transcript levels, and phenotype may be mediated through mitochondrial dysfunction due to elevated levels of *CYC1* expression (a gene which is expressed during mitochondrial expansion) and depletion of the nicotinamide adenine dinucleotide, reduced dehydrogenase subunit 1 mitochondrial gene.

However, there may be other gene effects that account for phenotypic abnormalities. AGG trinucleotide interruptions (typically separated by 9–11 CGG repeats) within the CGG-repeat element of the *FMR1* gene are known to disrupt the otherwise pure CGG-repeat motif. Normal *FMR1* alleles typically possess two or three AGG interruptions, premutation alleles generally possess two or less interruptions, and larger premutation alleles tend to have fewer AGG interruptions. The loss of AGG interruptions is thought to increase the probability of transmission of a full mutation allele.[51] Some researchers postulate that a lack of AGG interruptions may increase the likelihood of neurological symptoms in premutation carriers, but this has not yet been studied formally. The role of a lack of AGG interruptions in relation to the phenotypes of the gray zone carriers is even less clear.

Is there a biologic advantage to carrying gray zone alleles?

In a study testing 10,587 healthy females, there was a significant increase in the transmission of an abnormal allele between 51–60 CGG repeats compared to transmission of a normal allele.[52] This increase in transmission was not seen in carriers of >60 CGG repeats. This intriguing result may suggest that there is a genetic advantage for the abnormal allele in this range and that there is non-random transmission of the abnormal allele to offspring. Earlier studies showed that in mothers who carried higher premutation alleles (typically >70 CGG repeats), the normal allele was transmitted more frequently.[53]

How do you counsel a patient with a gray zone expansion?

Although some guidelines for genetic counseling would suggest that there are no consistent associations between phenotypes and gray zone alleles,[54] presenting a more measured approach might be considered for individuals with smaller expansions. This could mean discussing data that suggest that phenotypes may be associated; additional studies in this area will provide more certainty. These neurological phenotypes include FXTAS-like signs, parkinsonism, and ataxia. Given the clear association of a lack of AGG interruptions with *FMR1* repeat expansion in subsequent generations (repeat instability), risk estimates of fragile X-associated disorders in families who carry smaller expansions (45–69 CGG) may be more accurate with characterization of the AGG structure in an individual patient.[55]

Implications

The knowledge concerning gray zone *FMR1* repeat expansions is increasing, but almost all questions related to the genotype are yet to be definitively answered. Recommendations for future research in the area of the *FMR1* gray zone include determining a consistent definition of the repeat size of the genotype in the literature (41–54 vs. 45–54), consistent definition of the name (intermediate vs. gray zone), defining the clinical ramifications of a repeat expansion, describing the underlying pathology and imaging of these repeat expansion carriers, and the consequences for families. This paper focused mostly on neurological issues in gray zone expansion carriers, but phenotypes in other realms that are seen in premutation carriers, such as psychiatric, cognitive, and endocrine, should be investigated as well. A multifaceted program of research, similar to those created for the larger expansion carriers, would be ideal to answer some of these key questions.

There are several barriers that need to be overcome in carrying out this work. Cascade testing in families with fragile X children will not typically yield gray zone expansion carriers, who may be great grandparents. Instead, researchers who do population screening will need to identify and recruit those individuals with gray zone expansions who were ascertained in order to pool enough affected individuals to carry out the needed research. Collaborating with other researchers, which may include experts outside of neurology, who do screening or epidemiologic studies, will be critical to reach needed sample sizes. This will allow the field to move beyond case studies or clinic-based methods of study. Genetic counselors will also need to be educated to refer patients who are identified with gray zone expansions in order to facilitate local recruitment for these studies. Finally, recruiting more physicians who specialize in adult diseases into fragile X-associated disorders research will enhance studies in these patients, as symptoms are more likely to manifest in adult years similar to the premutation carriers.

In the past, with the discovery of FXTAS, research regarding the premutation rapidly progressed. It is the hope of many of the clinicians and researchers in the field that similar resources will enable quick resolution of some of the issues raised in this paper. Fragile X research is a highly collaborative enterprise, with many of the large centers and clinics working across institutions, disciplines, and countries. This is likely to speed discovery and, hopefully, enhance the likelihood these issues will be resolved in a timely manner.

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