# EXPLORING GENETIC COUNSELORS' PERCEPTIONS AND UTILIZATION OF AGG ANALYSIS TO REFINE FMR1 GENE EXPANSION RISK

by

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ABSTRACT

Newer testing methodology allows genetics service providers to refine and personalize an at-risk individual's chance to have a child with Fragile X syndrome, by determining the number and location of AGG insertions within the *FMR1* gene. This exploratory study aimed to better understand genetic counselors' knowledge, utilization, and attitudes towards the clinical utility of AGG analysis for their patients. Study investigators were under the impression counselors were not commonly integrating AGG analysis into their clinical practice. The hypothesis for this study was that genetic counselors were reserved in their uptake of this new testing technology due to limited knowledge, lack of perceived importance in care, and concern for cost burden to patients. To better determine reasons for limited uptake, current members of the National Society of Genetic Counselors, the Australasian Society of Genetic Counsellors, and the Association of Genetic Nurses and Counsellors were asked to participate in an online survey. Few survey respondents reported actually offering AGG analysis to their patients. The majority desired more education before they would feel knowledgeable enough to discuss this test with patients. Those with greater self-reported knowledge of AGG analysis were significantly more likely to discuss this testing option during counseling. By and large, counselors perceived AGG analysis to be important to care and relevant to their patients. This, in conjunction with other

results of the study, negates the hypothesis that lack of perceived importance in care has influenced low uptake. Even in the midst of insurance concerns, there was a willingness amongst counselors to consider offering this testing in the future. This study found a high level of desire among the participants for the establishment of formal testing guidelines by a governing organization, such as the National Society of Genetic Counselors or the American College of Medical Genetics. Information gathered from this study will be of benefit to the genetic counseling profession and may impact the utilization of genetic testing methodologies in the future. This research is of particular significance to the field of public health as Fragile X syndrome is the most common inherited cause of intellectual disability and autism.

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## PREFACE

This master's thesis was made possible through the collaborative efforts of several parties. Firstly, I am indebted to my thesis committee chair and program co-director, Elizabeth Gettig, MS, LCGC. Her continual guidance, support and friendship served to foster my growth as a genetic counseling student. Furthermore, she spent countless hours helping me to select a thesis project that would engage my interests and be relevant to my genetic counseling colleagues. Her support during those early stages and later her assistance as my thesis committee chair was invaluable.

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The statistical analysis was performed under the guidance of my committee member Daniel Normolle, PhD from the Department of Biostatistics. I am grateful to Dr. Normolle for being consistently available and willing to answer questions. His approach to mentorship

ensured I took full part in the analysis, which helped make this an especially educational opportunity.

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I am thankful to all of the genetic counselors that took part in this study. Finally, my whole-hearted thanks go out to my husband, family, and classmates. Your love, encouragement, prayers and support made all the difference.

# 1.0 INTRODUCTION

Fragile X syndrome is the most common inherited cause of intellectual disability and autism in both boys and girls. The disorder results from an expansion of a trinucleotide repeat (CGG) within the X-linked, Fragile X Mental Retardation-1 (*FMR1)* gene. An individual with greater than 200 CGG repeats, can show symptoms of the syndrome. In comparison, individuals in the general population commonly carry between 6 and 50 repeats (Sherman, 2000). This disorder is complicated by remarkable repeat instability and risk for expansion during oogenesis and post-zygotic mitosis (Brenda Finucane et al., 2012; Warren & Nelson, 1994). The sex of the parent carrying the expanded repeat, the number of CGGs, and the number and position of AGG insertions influence the risk for expansion in the next generation (Yrigollen et al., 2012).

Genetic testing has been available for decades to help predict risk for Fragile X syndrome in offspring. Historically, these predictions were based solely on the number of maternal CGG repeats. A newer test, that has been clinically available since 2011, enumerates AGG insertions within the CGG repeats. AGG insertions have long been shown to stabilize the *FMR1* gene and therefore help protect against repeat expansions. By determining the number and position of AGG insertions, this new testing methodology can refine and personalize an at-risk individual's chance to have a child with Fragile X syndrome. Ideally, this additional information can aid genetic counselors in helping women to make informed decisions about family planning in regards to both pre- and post-conception options.

At present, there are no formal testing guidelines to direct genetic counselors when to offer AGG analysis to refine risk for expansion. This research is an effort to better understand genetic counselors' knowledge, utilization, and attitudes towards the clinical utility of AGG analysis for their patients. The research will further serve to assess the desire for formal testing guidelines. Information gathered from this study will be of benefit to the genetic counseling profession and may impact the utilization of genetic testing methodologies in the future.

## 1.1 HYPOTHESIS AND SPECIFIC AIMS

### 1.1.1 Hypothesis

Genetic counselors may be reserved in their uptake of new testing technology for Fragile X carrier status due to limited knowledge, lack of perceived importance in care, and concern for cost burden to their patients. The establishment of formal testing guidelines by a governing organization would be a desirable resource to these healthcare professionals.

### 1.1.2 Specific Aims

**Aim 1:** Survey genetic counselors in the United States and abroad to characterize demographics, experience with counseling *FMR1* intermediate and/or premutation carriers, and perceptions and utilization of AGG analysis.

**Aim 2:** Explore perceived barriers to offering AGG analysis by investigating associations between survey variables, including current knowledge of AGG analysis and its impact on testing perceptions and utilization.

**Aim 3:** Ascertain genetic counselors level of desire for the establishment of formal testing guidelines by a governing organization.

## 1.2 BACKGROUND AND SIGNIFICANCE

### 1.2.1 Fragile X Syndrome

Fragile X syndrome (FXS) is the most common inherited cause of intellectual disability and autism in both boys and girls. It is estimated to affect ~1 in 4,000 males and ~1 in 8,000 females (Crawford, Acuña, & Sherman, 2001). While females with Fragile X syndrome generally have milder features than males, they can also exhibit a similar range of characteristics (McConkie-Rosell et al., 2005). In addition, variability in both sexes can result from mosaicism either in DNA methylation or repeat size (Warren & Nelson, 1994). The physical, cognitive and behavioral phenotype is therefore best recognized as a continuum with variable expressivity.

Physical characteristics can include long narrow face, prominent ears, prominent forehead or jaw, macroorchidism in male teens/adults, high arched palate, and pes planus. Several physical features become more visible over time. Due to the subtlety of facial dysmorphism, this syndrome can easily go unrecognized based on clinical presentation alone (Warren & Nelson, 1994). Individuals with FXS may also experience strabismus, recurrent otitis

media, hyperextensible joints, epilepsy and mitral valve prolapse (B. Finucane, McConkie-Rosell, & Cronister, 2002).

Cognitively individuals can encounter mild to severe developmental delay, learning and intellectual disabilities. Behavioral concerns can include attention deficit hyperactivity disorder (ADHD), hand flapping and/or biting, poor eye contact, and social anxiety. In addition, individuals with FXS can have speech and language delays and often exhibit rapid, repetitive speech. Many behavioral traits have significant overlap with autistic spectrum disorders, including difficulty with transitions and avoidance behaviors due to increased sensitivity to sounds, touch, crowds, certain foods and textures (B. Finucane et al., 2002). It has been estimated that as many as 30% of males with FXS meet formal diagnostic criteria for autism (R. Hagerman et al., 2009).

#### 1.2.1.1 Treatment and Management

At present, there is no cure for FXS. Treatments and interventions are supportive in nature to help individuals reach their full potential. All individuals are eligible for early intervention services (Crawford et al., 2001). Other treatments include the integration of occupational, physical, speech and language therapies into a special education program. In addition, some individuals benefit from behavioral interventions and medications to help manage their hyperactivity, poor attention span and/or other behavioral and emotional problems. As each child and adult with FXS can have a different presentation, it is beneficial to provide tailored care based on the individuals strengths and weaknesses (Brenda Finucane et al., 2012).

### 1.2.2 Molecular Genetics

Fragile X syndrome is one of several disorders caused by mutations in the Fragile X Mental Retardation-1 (*FMR1)* gene. The *FMR1* gene is located on the X-chromosome at position Xq27.3 and codes for the Fragile X Mental Retardation Protein (FMRP), which is necessary for pre- and postnatal brain development (Crawford et al., 2001). This trinucleotide repeat disorder results from an expansion of CGG repeats within the *FMR1* gene (Crawford et al., 2001). It is now understood that CGG expansion occurs in the 5' untranslated region of the first exon. A repeat expansion of greater than 200 CGGs leads to hypermethylation of the promoter region and suppresses transcription (Eichler et al., 1994). The methylated gene is therefore unable to make its FMRP protein product, which is normally abundant in neurons and expressed in a variety of other tissues (McConkie-Rosell et al., 2005).

Fragile X syndrome is inherited as an X-linked disorder with reduced penetrance. As with any X-linked disorder, a mother has a 50% chance to pass on her affected X chromosome to each of her children and a father will pass on his X chromosome to all of his daughters and none of his sons. The sex of the parent carrying the expanded repeat, the number of CGGs, and the number and position of AGGs influence the risk for expansion in the next generation (Yrigollen et al., 2012). A 2012 study assessed the contribution of maternal age and found that it was not a statistically significant contributor to expansion risk (Yrigollen et al., 2012).

Rarely, Fragile X syndrome can be the result of a point mutation or deletion of all, or a portion, of the *FMR1* gene. This accounts for fewer than 1% of individuals with FXS (McConkie-Rosell et al., 2005). However, because sequencing of *FMR1* is not routinely practiced this is less likely to be picked up outside of a research setting (Crawford et al., 2001).

#### 1.2.2.1 History

Prior to the discovery of the *FMR1* gene, early Fragile X researchers made the observation that penetrance in these families appeared to increase with successive generations. Researchers were able to make educated hypotheses about the risk for female carriers to have an affected child based on their placement within the pedigree. This was called the Sherman paradox (Sherman et al., 1985). In 1991, the *FMR1* gene was identified and further research uncovered a correlation between increasing number of CGG repeats and proclivity for disease. (Fu et al., 1991; Heitz, Devys, Imbert, & Kretz…, 1992). As the expanded allele is passed on through a family it tends to increase in size, which explains the greater number of affected children in later generations (Warren & Nelson, 1994). Resolution to the Sherman paradox came with the recognition of the phenomenon termed anticipation, whereby the number of affected individuals increases in subsequent generations.

The molecular mechanism of Fragile X provided the first evidence that not all disease-causing mutations are stably transmitted (Orr & Zoghbi, 2007). Interestingly, while these unstable trinucleotide repeats were first described in Fragile X syndrome, trinucleotide instability is not isolated to FXS. Over the next three years, unstable trinucleotide repeats were observed in several other neurological disorders such as Huntington disease, Spinocerebellar ataxia type 1, Kennedy's disease and Myotonic dystrophy. Each of these disorders is further characterized by anticipatory inheritance. The discovery of trinucleotides made a lasting impact on research related to mental retardation, Huntington disease, inherited ataxias and muscular dystrophy. As of 2007, these expansions were known to account for at least 16 neurological conditions (Eichler et al., 1994; Orr & Zoghbi, 2007).

Historically, predicting the risk of CGG repeat expansion in offspring has been of considerable difficulty. This is particularly true for women with alleles in the intermediate range (45 to 54 repeats), which overlap the high-end of that found in the general population and the low end of that found in Fragile X families (Fu et al., 1991; Warren & Nelson, 1994). This difficulty in predicting expansion was illuminated by studies that identified stable and unstable alleles of similar size. This suggested that a feature other than repeat length must also be involved in stability (Eichler et al., 1994). Other research noted that repeat size seemed to be more similar among siblings than among unrelated patients. This too suggested a factor outside of repeat length must play a role in expansion. These observations were particularly relevant to genetic counselors, as the risk to have a child with Fragile X syndrome could significantly differ even amongst women with the same repeat size (S. Nolin et al., 1996).

In the mid-1990s, researchers observed AGG triplets interspersed within the CGG repeat tract. It was soon hypothesized that the number and position of interrupting AGGs were involved in stability and their loss contributed to disease predisposition (Warren & Nelson, 1994). Supporting studies found that most *FMR1* alleles possessed two interspersed AGG interruptions, whereas those shown to have zero or one had an increased likelihood of unstable transmissions. A 1994 study, suggested that 34 to 37 consecutive CGGs were a threshold for repeat instability, with larger repeat numbers conferring a higher likelihood of expansion. The presence of AGG trinucleotides suggested enhanced stability and instability was thought to result from their loss (Eichler et al., 1994). Follow-up studies investigated the smallest repeat lengths to expand to a full mutation in one generation. They found that these alleles, containing 56 and 59 CGGs, had zero AGG interruptions. This served to underscore the probable role of AGG

interruptions in providing intergenerational repeat stability (Fernandez-Carvajal et al., 2009; Sarah Nolin et al., 2003).

Researchers concluded the loss of AGG was an important mutational event in predisposing individuals to Fragile X syndrome (Eichler et al., 1994). The precedence for this model was established for Spinocerebellar ataxia type 1, in which all disease alleles exhibit loss of CAT interruptions from the CAG trinucleotide repeat (Chung et al., 1993). With other triplet repeat conversions in mind, later researchers proposed that the loss of AGG interruptions could be the result of an A to C transversion or a deletion (Eichler et al., 1994). Furthermore, it was postulated that the loss of AGG interruptions resulted in an increased rate of DNA slippage (Zhong et al., 1996). Unfortunately, come present day the molecular basis of the "AGG effect" has still not been fully elucidated (Yrigollen et al., 2012).

#### 1.2.2.2 CGG Repeat Length

The length of CGG repeats can be divided into four categories: stable (6 to 44), intermediate (45 to 54), premutation (55 to 200) and full mutation (>200). The categorization of repeat lengths is somewhat arbitrary, as different studies have used varying repeat length cut-offs. To address these ambiguities the American College of Medical Genetics operationally defined these categories based on size of CGG repeat, irrespective of the presence or absence of AGG interruptions (Maddalena et al., 2001). The influence of repeat size on possible clinical features and likelihood of expansion can be seen in Table 1.

**Table 1.** Influence of CGG Repeat Size

| Category | Repeat Size | Clinical Features | Likelihood of Expansion |
|---|---|---|---|
| Stable | 6 to 44 | None | Stable |
| Intermediate | 45 to 54 | None | Small likelihood—usually only by a few repeats |
| Premutation | 55 to 200 | Fragile X-associated primary ovarian insufficiency; Fragile X-associated tremor/ataxia syndrome | Unstable—may expand to full mutation with maternal transmission |
| Full mutation | >200 | Fragile X syndrome | Unstable |

Stable *FMR1* alleles are common among the general population and are usually transmitted from parent to offspring in a stable manner (Crawford et al., 2001). Most individuals have thirty CGG repeats, with AGG interruptions occurring most often at positions 10 and 20 (Sarah Nolin et al., 2003; S. Nolin et al., 1996).

Alleles with 45 to 54 CGG repeats fall into the gray zone or intermediate range. Intermediate alleles show variable stability but have not been shown to expand to a full mutation within one generation (Fu et al., 1991). The smallest known single-generation expansion to a full mutation is 56 repeats. Interestingly in that case study, the maternal grandfather was a carrier of an intermediate allele with 52 repeats. This is demonstrative of an intermediate allele expanding to a full mutation within two generations (Fernandez-Carvajal et al., 2009).

Premutation alleles are considered extremely unstable in meiotic transmission and may expand to a full mutation in one generation. In addition, they have been shown to be mitotically unstable, as mosaicism has been observed on numerous occasions (Fu et al., 1991). Premutation alleles mutate virtually every time males or females pass them on. This transmission most often

results in an increased CGG repeat length. In fact, nearly all those who inherit a maternal premutation (98.7%) carry a larger repeat size than their mother (S. Nolin et al., 1996). These premutation alleles will eventually expand to a full mutation via maternal transmission. The risk for expansion has been shown to intensify with increased CGG repeat length (Sarah Nolin et al., 2003). For example, maternal alleles with >90 repeats almost always expand to a full mutation within one generation (Crawford et al., 2001). Importantly, individuals with a premutation allele do not typically show symptoms of FXS, though they can face unique alternate health problems (Warren & Nelson, 1994).

Finally, the full mutation is defined by a combination of repeat expansion (>200 CGG repeats) and DNA methylation. Remarkably, some individuals have more than 1,000 repeats. This massive expansion in repeats causes the *FMR1* gene to become methylated inducing transcriptional silencing. While all males with a full mutation are affected with FXS, only approximately half of females show symptoms. This reduced penetrance in females can largely be attributed to X-inactivation (Warren & Nelson, 1994). Other phenotypic variability in both sexes can be attributed to methylation status and mosaicism (Brenda Finucane et al., 2012). A study done in 1992, found ~15% of subjects with a full mutation were in fact mosaics with some cells containing premutation alleles (Heitz et al., 1992). This finding is indicative of mitotic instability in the full mutation. While women with a full mutation (>200 repeats) can pass on a full mutation to their children, paternal transmission of the full mutation is rare. Men with a full mutation typically only pass on a premutation allele to their daughters. This is theorized to be due to differences in the male germ line, perhaps because of selection against the full mutation in sperm (Crawford et al., 2001; S. Nolin et al., 1996).

#### 1.2.2.3 Predicting Risk for Expansion

In 2006, the National Society of Genetic Counselors established a task force charged with updating the definition of genetic counseling (National Society of Genetic Counselors' Definition Task et al., 2006). This group of experts carefully constructed the below definition.

> *Genetic counseling is the process of helping people understand and adapt to the medical, psychological and familial implications of genetic contributions to disease. This process integrates the following:*
> - *Interpretation of family and medical histories to assess the chance of disease occurrence or recurrence.*
> - *Education about inheritance, testing, management, prevention, resources and research.*
> - *Counseling to promote informed choices and adaptation to the risk or condition.*

In alignment with this definition, it is of upmost importance for genetic counselors working with families at-risk to have a child with an *FMR1* mutation, to provide accurate assessments of likelihood. However, an accurate evaluation of occurrence or recurrence in a family is dependent on the technology available. In order to promote informed choices among women who are weighing the risk of having a child with Fragile X syndrome, counselors need to continually stay informed of the benefits and limitations of new testing technology.

In 2003, maternal CGG repeat sizes were collected in an effort to clarify the risk for expansion to full mutation. The researchers were cautious in interpreting risk estimates due to the possible influence of ascertainment bias from including only Fragile X families. This method theoretically could have excluded stable alleles in the same size category (Sarah Nolin et al., 2003). In 2011, Nolin et al. confirmed that expansion risk appeared higher for women with a family history of FXS as compared to those without. Through a prospective systematic analysis of over 1,000 prenatal samples, their group was able to refine the risks for expansion by maternal repeat size (Sarah Nolin et al., 2011). The risks from both studies are outlined in Table 2.

**Table 2.** Risk for Expansion to Full Mutation by Maternal Repeat Size

| | Study by Nolin et al. 2003 | | Study by Nolin et al. 2011 | |
|---|---|---|---|---|
| Maternal Repeat Size | % Expanding to Full Mutation | No. of Transmissions | % Expanding to Full Mutation | No. of Transmissions |
| 55 to 59 | 3.7% | 1/27 | 0% | 0/86 |
| 60 to 69 | 5.3% | 6/113 | 2.5% | 2/81 |
| 70 to 79 | 31.1% | 28/90 | 31.9% | 15/47 |
| 80 to 89 | 57.9% | 81/140 | 73.8% | 45/61 |
| 90 to 99 | 80.2% | 89/111 | 93.9% | 31/33 |
| 100 to 200 | 98.5% | 194/197 | 97.9% | 93/95 |

In most regards the 2011 study seemed to corroborate the findings from 2003. Of significance, the risk for expansion to a full mutation from a maternal repeat size of 55 to 59 seemed to be substantially less than previously thought. In addition, the updated risk for expansion was higher for mothers with 80 to 100 repeats (Sarah Nolin et al., 2011).

For a time, the percentages outlined above were the most accurate numbers available to genetic counselors providing risk assessment. Although researchers were aware that expansion was influenced by the absence of normally interspersed AGG triplets, the contribution of AGGs to this expansion risk was not quantified until 2012. At that time, Yrigollen, et al. retrospectively analyzed 267 premutation alleles, accounting for almost 400 transmission events. The group collected data on maternal CGG repeat lengths, the number and position of AGG interruptions, and repeat lengths in offspring. Their results supported previous findings that AGG interruptions decreased the chance of expansion to a full mutation. Yrigollen, et al. felt the results were significant enough to conclude "that failure to account for AGG interruptions can result in

profound errors in predicted risk for fragile X syndrome". Furthermore, their group believed AGG analysis was imperative to accurate assessments of risk for mothers who are premutation carriers (Yrigollen et al., 2012).

Yrigollen et al. demonstrated that AGG interruptions at least partially reduced expansion risk for all maternal premutations below ~100 CGGs. However, AGG interruptions seemed to have the greatest influence in risk prediction for alleles with 70-80 CGG repeats, as seen in Figure 1. In this repeat range, difference in risk varied by ~60% depending on number of AGGs present. As an example, the risk for expansion to a full mutation for a repeat length of 75 CGGs was 77% for alleles with no AGGs, as compared to 12% for alleles with two AGGs (Yrigollen et al., 2012).

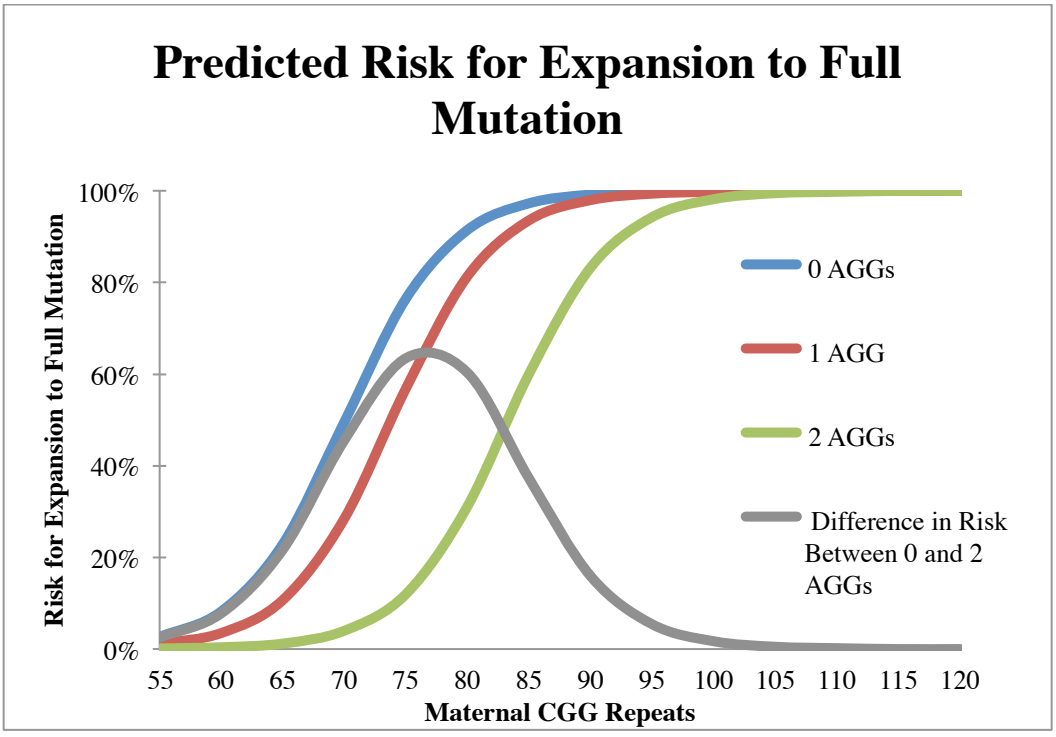


**Figure 1.** Predicted Risk for Expansion to Full Mutation

*Adapted from (Yrigollen et al., 2012)

Another 2012 study analyzed the impact of AGG insertions on small premutation alleles with 45-69 CGG repeats. Their results demonstrated the use of AGG analysis to differentiate small premutation alleles at the greatest risk for instability. In particular, they observed a clear risk to have a child with a full mutation for women carrying alleles of greater than 54 repeats and lacking AGG insertions. The authors felt the risks posed to these women warranted the option of prenatal diagnosis (Sarah Nolin et al., 2013).

### 1.2.3 Other FMR1-Related Disorders

It was initially suspected that carriers of the unstable premutation allele did not have phenotypic consequences. Researchers theorized that because these alleles were unmethylated and transcriptionally active, they produced expected levels of protein product, FMRP (Sherman, 2000). While some premutation carriers do not have associated health problems, research has uncovered that many present with a spectrum of physical, cognitive, and behavioral findings. These can range from mild features of Fragile X syndrome, to fragile X-associated primary ovarian insufficiency, to fragile X-associated tremor/ataxia syndrome (McConkie-Rosell et al., 2005). Therefore, the identification of a proband with Fragile X syndrome can easily lead to the recognition of multigenerational family involvement (R. Hagerman et al., 2009).

#### 1.2.3.1 Fragile X-associated Primary Ovarian Insufficiency

In 1991 researchers, such as Cronister et al., began to investigate a perceived earlier onset of menopause amongst heterozygous carriers. Their study was performed prior to the categorization of women as pre- or full mutation carriers. Interestingly, they found 13% (8/61) of non-impaired carriers had onset of menopause before the age of 40, as compared to only 5%

among the control group (Cronister et al., 1991). Future studies were able to delineate pre- and full mutation carriers, showing women who carried the premutation were at the increased risk for ovarian dysfunction. These women were at as high as a 21% (95% confidence interval: 15-27%) risk for cessation of menses before the age of 40. Surprisingly, women with a full mutation were similar to the general population risk, 1% (Sherman, 2000). It was unclear whether variable expressivity or reduced penetrance played a role for the female premutation carriers not experiencing early menopause. Furthermore, age at menopause did not seem to be correlated with specific repeat size or X-inactivation pattern. Studies went on to suggest that subtle changes in FMRP expression might influence ovarian function. Researchers have since proposed that the absence of primary ovarian insufficiency in women with the full mutation relates to mRNA production. Women with the premutation allele produce the transcript with a large repeat tract, whereas full mutation carriers exclusively produce mRNA from their normal allele (Sherman, 2000).

#### 1.2.3.2 Fragile X-associated Tremor/Ataxia Syndrome

Fragile X-associated tremor/ataxia syndrome is another relevant *FMR1*-related disorder. This progressive neurodegenerative condition is estimated to effect ~46% of aging male and 17% of aging female premutation carriers (Rodriguez-Revenga et al., 2009). Symptoms can include intention tremor, gait ataxia, peripheral neuropathy, autonomic dysfunction, brain atrophy and cognitive decline (P. Hagerman & Hagerman, 2004).

#### 1.2.3.3 Other Premutation-associated Issues

The penetrance of premutation pathologies in Fragile X syndrome families was investigated in the late 2000s. Amidst noting premature ovarian insufficiency to be present in

almost one-fifth (19%) of women, they also found thyroid disease and chronic muscle pain to be fairly common among adult women with premutation alleles (16% and 24% respectively) (Rodriguez-Revenga et al., 2009). An increased frequency of autoimmune disorders, such as hypothyroidism and fibromyalgia, has also been observed (R. Hagerman et al., 2009; McConkie-Rosell et al., 2005). Finally, investigators observed higher than expected rates of mental health issues, hypertension, tremor and neuropathy in these adult women (Brenda Finucane et al., 2012; P. Hagerman & Hagerman, 2004).

### 1.2.4 Testing

There have been a myriad of tests available to patients and their families both prior to and after the *FMR1* gene discovery. Almost all males with Fragile X syndrome, and some affected females, exhibit an inducible fragile site at the distal end of the X chromosome. Prior to 1991, chromosome analysis for this cytogenetic marker, FRAXA, was a commonly used diagnostic test (Warren & Nelson, 1994). Researchers Sutherland and Jacky were at the forefront of investigations to describe the conditions of tissue culture needed to induce fragile sites (Hecht, Jacky, & Sutherland, 1982; Jacky, Beek, & Sutherland, 1983). However, this test method was not able to detect carriers and often delivered inconclusive or inaccurate results (Warren & Nelson, 1994). The inducible FRAXA fragile site eventually proved valuable in the identification of the gene itself (Crawford et al., 2001).

Carrier testing was initiated in the late 1980's and early 1990's with the usage of DNA linkage studies. While these studies were costly and time-consuming, they could be as high as 99% accurate in some families. Following the *FMR1* gene discovery, testing became even more reliable with direct DNA analysis (B. Finucane et al., 2002). Southern blots, in combination with

restriction enzymes, quickly became a popular and accepted method for DNA-based testing. This allowed for visualization of CGG repeat expansion and determination of methylation status for an individual. This labor intensive test requires a large amount of DNA (Crawford et al., 2001).

DNA diagnostic tests using polymerase chain reaction (PCR) are inexpensive, automated and fast. PCR can be performed on much smaller amounts of DNA and can typically distinguish alleles in the normal, intermediate, premutation and full mutation range. Various PCR protocols have equally variable sizing accuracies. Most laboratories take advantage of both Southern Blot and PCR to increase sensitivity to 99% for detecting affected and carrier individuals (Crawford et al., 2001; McConkie-Rosell et al., 2005).

At present, Asuragen® is the only company offering AGG analysis on a clinical basis and has been since 2011. This CLIA certified laboratory uses a PCR-based approach, in combination with capillary electrophoresis, to determine the comprehensive *FMR1* genotype. They report both the location and number of AGG insertions within the CGG repeat tract, for both males and females ("Xpansion Interpreter®," 2014). This testing is almost always performed as a reflex test following determination of CGG repeat length. The clinical report provided by the laboratory delivers both a risk number and risk range using a 95% confidence interval. These numbers can be used to predict likelihood of expansion to a full mutation upon transmission to offspring.

According to a brochure published by Asuragen®, the following reasons are appropriate for AGG analysis, including *a)* known Fragile X carriers with 45-90 repeats; *b)* those with a family history of Fragile X, Fragile X-associated tremor/ ataxia syndrome, and/or Fragile X-associated primary ovarian insufficiency; *c)* those with a family history of unexplained intellectual disability, developmental delay or autism; and *d)* those with a personal or family

history of female infertility ("Xpansion Interpreter®: The Next Step in Fragile X Testing," September 2013).

#### 1.2.4.1 Prenatal Testing

DNA-based testing has enhanced the accuracy of prenatal testing. For at-risk couples identified to carry a pre- or full mutation, prenatal diagnosis can be performed using either chorionic villus sampling (CVS) or amniocentesis. Though rare, CVS results can be complicated by borderline methylation patterns and may need to be clarified with amniocentesis. Unfortunately, regardless of testing strategy, limitations abound as to predicting the clinical implications of a full mutation in a female fetus and/or the consequences of a premutation later in life. Couples at risk to have a child with an *FMR1* mutation have a variety of pre-conception options including gamete donation, adoption and pre-implantation genetic diagnosis to avoid the risk of passing on an expanded allele (Brenda Finucane et al., 2012; McConkie-Rosell et al., 2005).

#### 1.2.4.2 Population Screening

Population-based screening has been an evolving discussion in the United States. While there is evidence for racial and ethnic variability, studies suggest the prevalence of premutations in the Caucasian general population is ~1 in 1,000 for men and between 1 in 246 and 1 in 468 for women. Some groups feel this relatively high prevalence, coupled with recent technological advances in testing, make Fragile X syndrome amenable to screening (Crawford et al., 2001).

Some proponents have considered offering women of reproductive age preconception screening to inform carriers of their genetic status and reproductive risk. In fact, *FMR1* mutation testing is increasingly being offered to women who express interest even without any known risk

factors (Brenda Finucane et al., 2012). Others have debated the benefits and limitations of newborn screening, which has been investigated in research-based pilot studies (McConkie-Rosell et al., 2007; McConkie-Rosell et al., 2005). In addition, there have been numerous discussions about the potential use of *FMR1* mutation testing as a diagnostic tool for women with ovarian dysfunction. This type of testing could have a relatively high diagnostic yield, as approximately 13.8% of idiopathic familial premature ovarian failure can be uniquely attributed to the premutation allele (Sherman, 2000).

The common detection of intermediate alleles in screening for Fragile X carriers is of particular concern. According to a 1996 study, ~4.8% of males and 9.5% of females randomly selected from the general population will have an allele size of 40 to 60 CGGs. These intermediate alleles are particularly important to consider as they present difficult issues for genetic counseling (S. Nolin et al., 1996). When CGG repeats in this range are detected in the general population they become particularly difficult to interpret (McConkie-Rosell et al., 2005).

# 2.0 MATERIALS AND METHODS

This thesis project was reviewed by the University of Pittsburgh's Institutional Review Board and was determined to meet criteria for exemption (Appendix A).

## 2.1 PARTICIPANTS

This exploratory study sought the opinion of genetic counselors both nationally and internationally. Current members of the National Society of Genetic Counselors (NSGC), the Australasian Society of Genetic Counsellors (ASGC), and the Association of Genetic Nurses and Counsellors (AGNC) were invited to participate. The NSGC is comprised of approximately 3,000 members practicing across the United States and into Canada. The ASGC is made up of 271 members practicing across Australia and New Zealand. The AGNC has approximately 300 genetic professionals practicing in the United Kingdom.

## 2.2 INSTRUMENTATION

### 2.2.1 Recruitment E-mail

E-mails inviting genetic counselors to participate in an online survey were drafted for members of the NSGC, ASGC, and AGNC. A copy of each of these e-mails can be seen in Appendix B.2.1, Appendix B.2.2, and Appendix B.2.3, respectively. These e-mails served as an introduction for genetic counselors to the project and provided a link to access the survey on the Internet. An e-mail was sent out to all members of the NSGC on October 9, 2013 through an E-mail blast system called Constant Contact. A reminder e-mail went out to the same group on October 23, 2013. Members of the ASGC were emailed by their secretariat through a listserv, on November 24, 2013. Finally, the AGNC Vice-Chair emailed their members on October 23, 2013. In addition, several genetic counselors interested in this thesis project took the liberty of forwarding their own reminder e-mails to colleagues.

### 2.2.2 Recruitment Brochure

In order to help generate interest in this project, several copies of a recruitment brochure were distributed and posted at the NSGC 32nd Annual Education Conference held October 9-12, 2013, at the Anaheim Convention Center in Anaheim, California. This brochure was designed to generate interest in the project and provide a link to access the survey on the Internet. In addition, this served as a reminder for NSGC members who received the invitation e-mail on October 9th but had not yet completed the survey. A copy of this recruitment brochure can be seen in Appendix B.1.

### 2.2.3 Survey Design

The earliest draft of this survey was the collaborative effort of genetic counselors, Brenda Finucane, MS, LGC from Geisinger Health System and Amy Cronister, MS from Integrated Genetics. A copy of this survey's first draft from June of 2013 can be seen in Appendix C. This early draft was then circulated for feedback to four other well-respected genetic counselors with expertise in Fragile X including, Liane J. Abrams, MS, LGC from the National Fragile X Foundation, Alison D. Archibald, PhD, GDipGenetCouns from the Victorian Clinical Genetics Services, Robin L. Bennett, MS, CGC, D. Sc. Hon. from the University of Washington Medical Center, and Allyn McConkie-Rosell, PhD, CGC from Duke University Medical Center.

In July of 2013, the author was recruited as a genetic counseling graduate student to bring this research project to fruition as part of this thesis project. Together with the genetic counselors mentioned above, the survey was carefully evaluated several times as questions were added, removed and restructured. It was eventually reformatted and reorganized to create a seemingly more efficient structure. Statistician, Daniel Normolle, PhD, also evaluated this survey. Dr. Normolle helped to ensure the questions were unbiased and constructed in such a way as to be most likely to achieve statistical significance. The final version of the survey can be seen in Appendix D.

The survey introduction served to inform participants that the purpose of this research was to explore genetic counselors' perceptions and utilization of AGG analysis to refine the risk of expansion in the *FMR1* gene for their patients. There was minimal risk involved in the study, as all results generated through the electronic survey would be collected anonymously. However, given the nature of the topic, it was possible some questions could have caused distress as some individuals could have felt uncomfortable thinking about the ethical

implications of deciding whether or not to offer AGG testing to patients. It was stressed that participation in this anonymous survey was voluntary and individuals had the option to exit out of the survey at any time. There were no costs to participating nor were there direct benefits. The introduction highlighted that information gathered from this study would be of benefit to the genetic counseling profession and could impact the utilization of genetic testing methodologies in the future. Any questions were to be targeted to the primary investigator, LHB.

Participants were asked up to twenty-one questions. Demographic questions were used to assess factors such as, how long individuals had been in practice, in what specialty area they focused, whether or not they counseled patients, and if they had ever counseled patients with an *FMR1* intermediate or premutation allele. Additional questions were structured to gauge current knowledge level of AGG analysis and document how this education was obtained. Further questions sought to tease apart why genetic counselors were or were not offering AGG analysis and for whom. The final portion of the survey was designed to capture genetic counselors' level of desire for the establishment of formal testing guidelines.

## 2.3 PROCEDURES

### 2.3.1 Survey Distribution

For ease of distribution and data collection, the finalized survey was made available electronically through SurveyMonkey®. The electronic version of the survey differed only from that found in Appendix D due to its incorporation of skip logic. Skip logic helped streamline questions so that participants were automatically directed to the next appropriate question.

### 2.3.2 Results Collection

Responses were collected and analysis was initiated using features of SurveyMonkey®. The analysis section of the website displayed a full summary of all questions and responses, complete with charts and tables. The data could be displayed in a variety of other chart types as well. Analysis tools on this website further allowed the researchers to filter by question and answer as well as to compare questions side-by-side in a process known as cross tabulation. Finally, this website allowed administrators to export all response data into both Microsoft Excel® and IBM SPSS Statistics® for more in depth analysis.

## 2.4 STATISTICAL ANALYSIS

Statistical analysis of the quantitative and qualitative data was performed under the guidance of a statistician at the University of Pittsburgh. Data was exported from SurveyMonkey® into both Microsoft Excel® and IBM SPSS Statistics®. Much of the descriptive statistics were performed using the SurveyMonkey® analysis tools. These tools quickly tabulated answers and allowed the investigators to filter by question and answer as well as to compare questions side-by-side in a process known as cross tabulation. In IBM SPSS Statistics®, Fisher's exact test was performed on two-by-two tables to check for statistical significance for numerous comparisons. Of note, there were a few questions that asked participants to "fill-in-the-blank" with a number. Some individuals wrote estimations such as "1 to 2" years in practice or "more than 20" patients counseled. For a conservative approach to our statistical analysis these values were either recorded as the median of their answer or the lowest value (1.5 and 20, respectively).

# 3.0 RESULTS

## 3.1 NSGC RESPONDENT STATISTICS

### 3.1.1 Demographics

Two thousand nine hundred and thirty-two NSGC members were successfully e-mailed to participate in this research study. Five hundred and sixty-nine individuals opened the email, which accounts for 19.4% of all individuals that received the email. As a comparison, according to the NSGC Membership Associate most student research surveys have open rates between 13 and 22%. In total, three hundred and ninety members (13.3%) agreed to participate.

Of the 390 NSGC respondents, almost all (94.9%; n=370) reported working primarily in the United States. The rest worked in Canada (4.9%; n=19) and one counselor reported working in Australia. Approximately half (55.1%; n=215) of the counselors surveyed had been practicing for five years or less, as can be seen in Figure 2. The mean years of practice was 8.11 years (SD ± 8.69; range 0 to 39 years; n=390). The median was 4 years of practice.

The survey further elicited which specialty area(s) counselors practiced in. The majority (60.3%; n=235) of survey respondents reported specializing in prenatal genetics. Some of these counselors reported specializing in additional areas of genetics as well. Figure 3 shows the distribution of specialty area(s) by respondents. In the "Other" category, respondents reported

myriad specialties including neurology, ophthalmology, cardiovascular disease, metabolic disease, and infertility to name a few. Of the 44 counselors who marked "Other", most of them (59.1%; n=26) also identified with at least one of the provided answer choices.

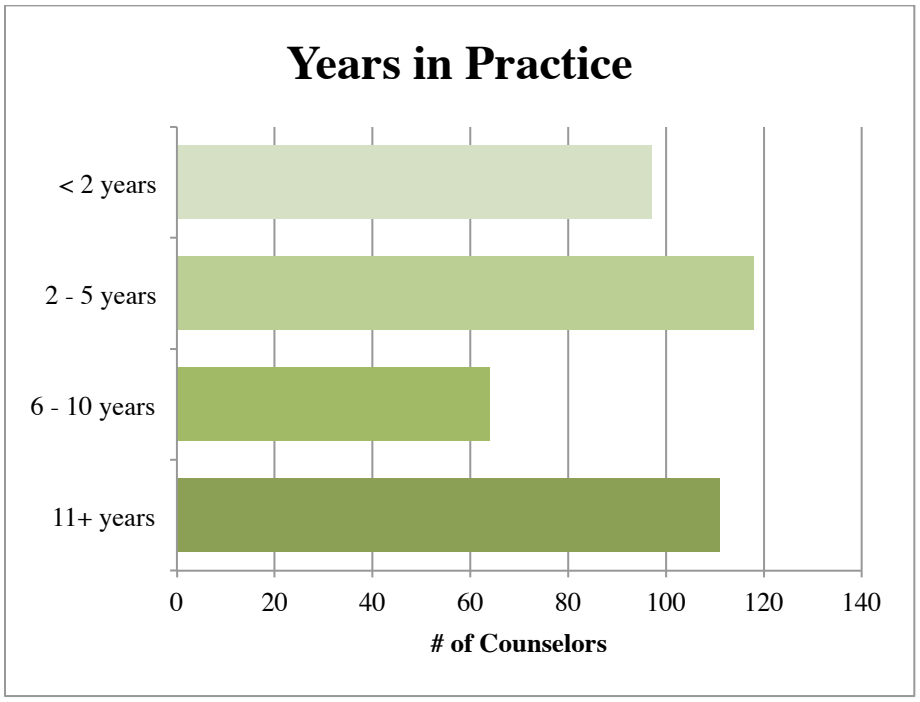


**Figure 2.** NSGC—Years in Practice

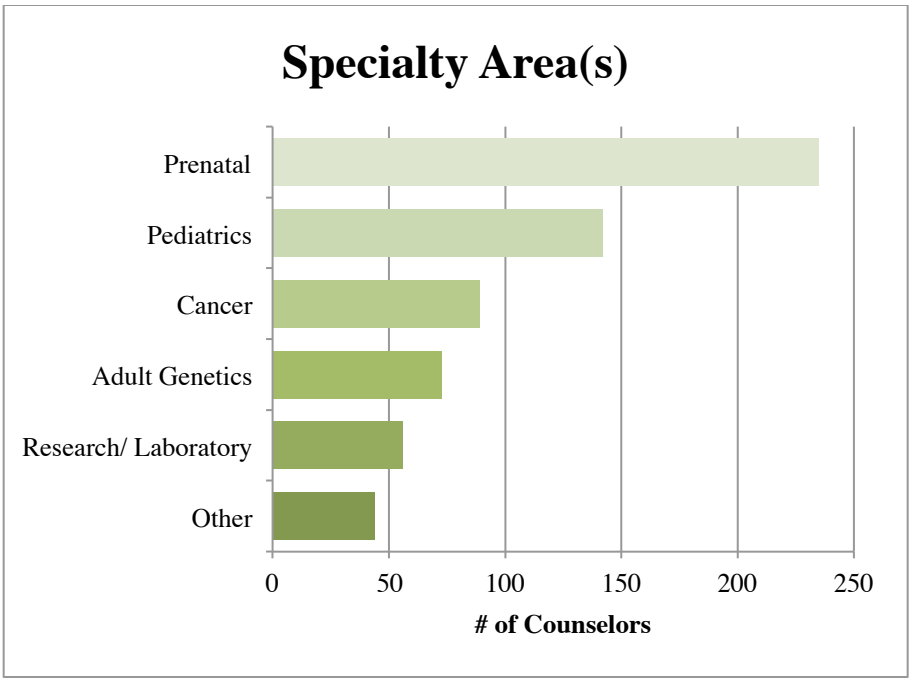


**Figure 3.** NSGC—Specialty Area(s)

*Respondents (n=390) were able to select multiple specialty areas.

### 3.1.2 Knowledge of AGG Analysis

In order to assess knowledge levels, counselors (n=386) were asked how familiar they were with the notion of analyzing AGG interruptions in women with an *FMR1* intermediate or premutation allele. The counselors were asked to rate their familiarity on a scale of 1 to 4, with 1 being not at all knowledgeable and 4 being very knowledgeable. Most counselors (81.9%; n=316) reported at least some degree of knowledge. However, only 11.7% of counselors (n=45) considered themselves very knowledgeable, as can be seen in Figure 4.

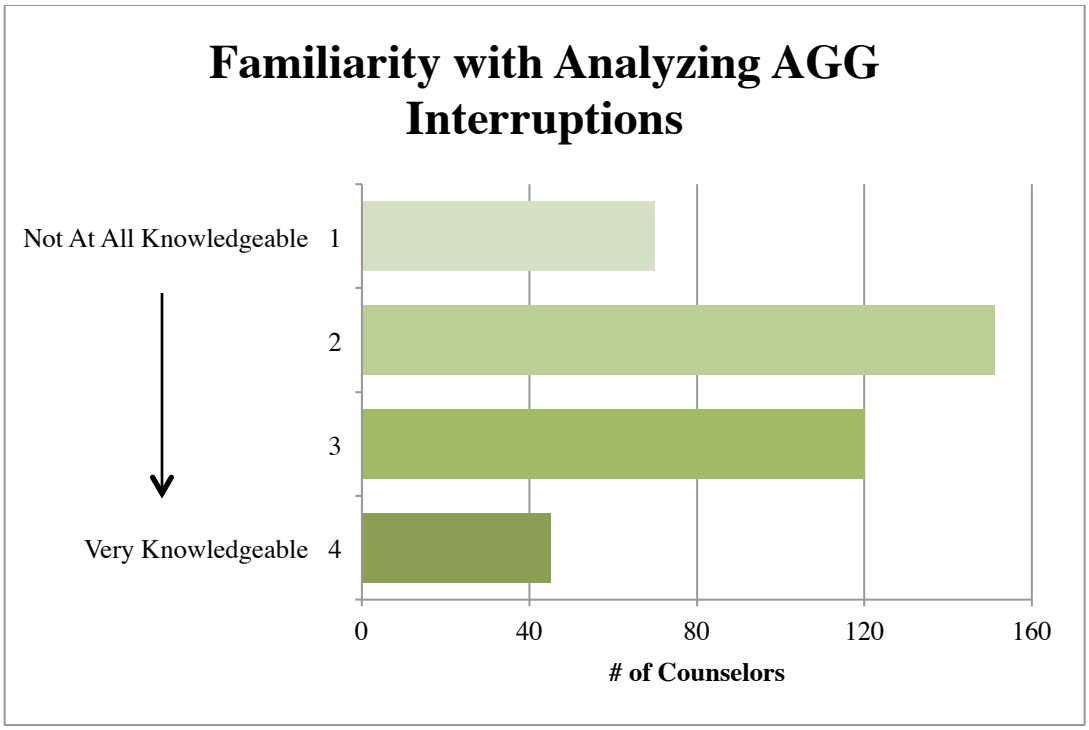


**Figure 4.** NSGC—Familiarity with Analyzing AGG Interruptions

*Respondents (n=386) were asked to rate their familiarity on a scale from not at all knowledgeable (1) to very knowledgeable (4).

Amongst the counselors with at least some degree of knowledge (rating themselves 2, 3 or 4), education about AGG analysis was obtained in a wide array of methods. Out of the 313 counselors answering this question, all with at least some degree of knowledge, almost half (47.9%; n=150) reported reading peer-reviewed articles on the studies performed to date. Many also reported attending lectures/presentations on AGG analysis and/or reading promotional materials from a company or institution that performs the testing. In response to this question, a few individuals (n=33) added additional comments about where they obtained their knowledge. The most often cited additional sources were discussions with colleagues, reading GeneReviews™, in genetic counseling training programs, and while attending NSGC's Annual Education Conference. The breakdown of education method can be found in Figure 5.

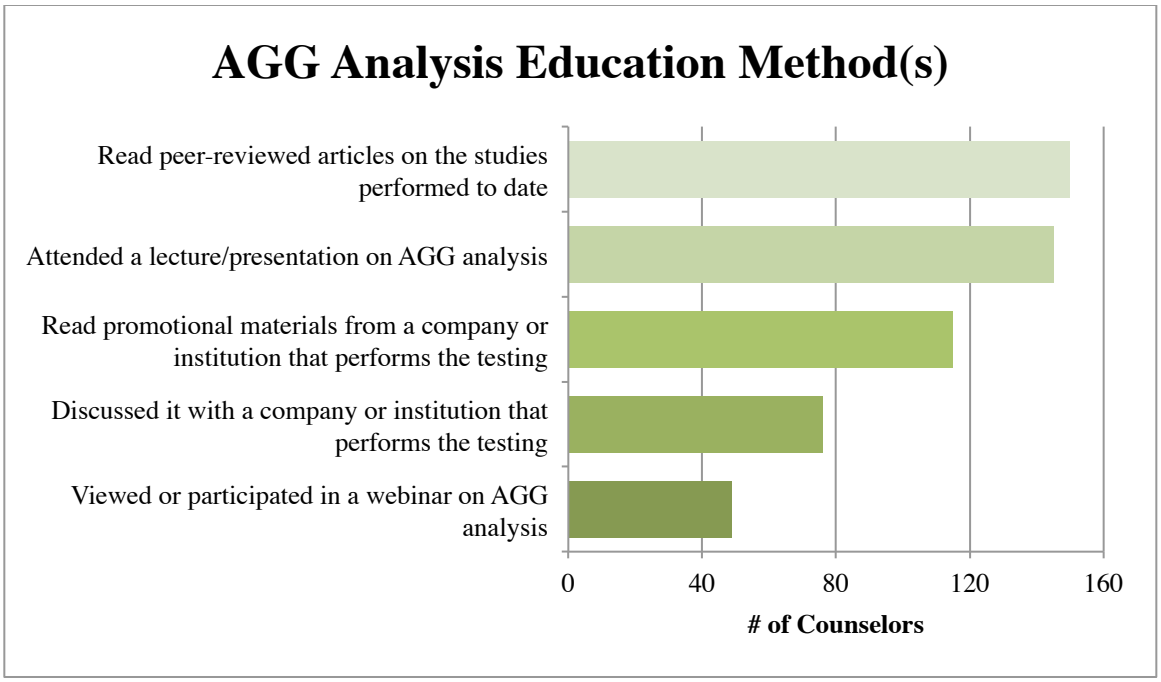


**Figure 5.** NSGC—AGG Analysis Education Method

*Respondents (n=313) were able to select multiple methods.

### 3.1.3 FMR1 Patient Experience

Of the 390 respondents, most (92%; n=360) reported that they counsel patients. Further, the majority of counselors taking part in this survey (79.0%; n=308) have counseled a patient with an *FMR1* intermediate or premutation allele. Polling these 308 individuals, they have on average each counseled ~6.4 patients with an *FMR1* intermediate or premutation allele over the past 3 years (SD ± 11.6; range 0 to 100). The median number of patients counseled with an *FMR1* intermediate or premutation allele over the past 3 years was 3. The distribution of *FMR1* patients counseled over the past 3 years is shown in Figure 6.

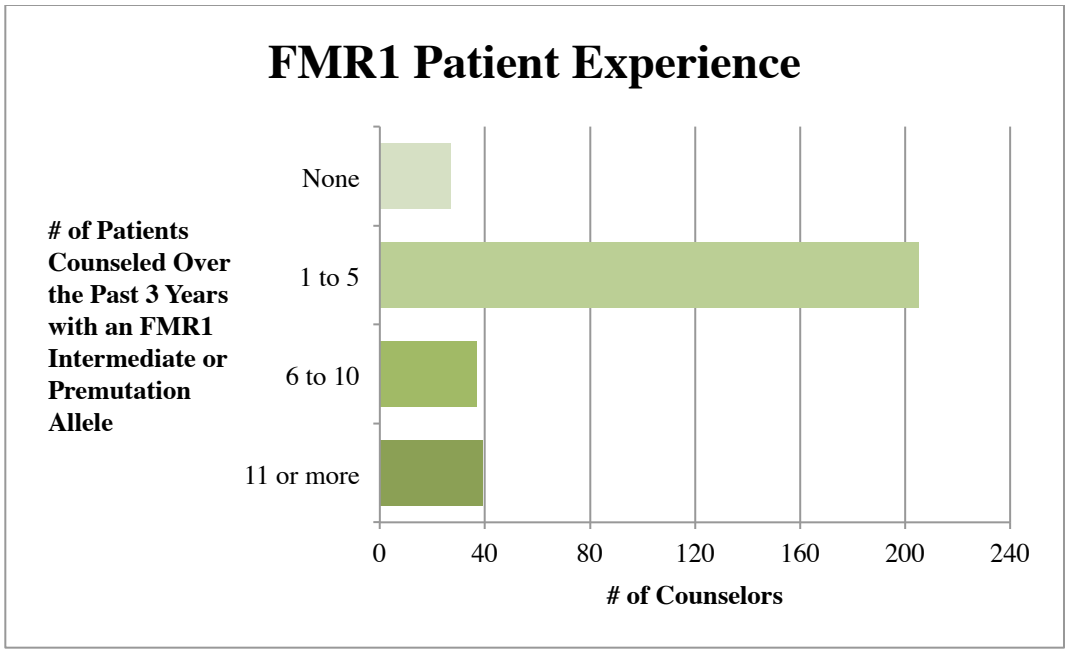


**Figure 6.** NSGC—*FMR1* Patient Experience

Counselors were further asked how often they discuss the role of AGG analysis when counseling patients with an *FMR1* intermediate or premutation allele. About a quarter of the respondents (28.5%; n=102) felt this question was not applicable to them, likely because they do not counsel patients for this indication. Of the remaining 256 counselors, the majority (58.2%; n=149) "never" discussed the role of AGG analysis, while only 13.3% (n=34) reported "always" discussing AGG analysis. Interestingly, counselors who considered themselves to be more knowledgeable about AGG analysis were more likely to discuss this topic during counseling (p-value = <0.0001), as can be seen in Figure 7 and Table 3.

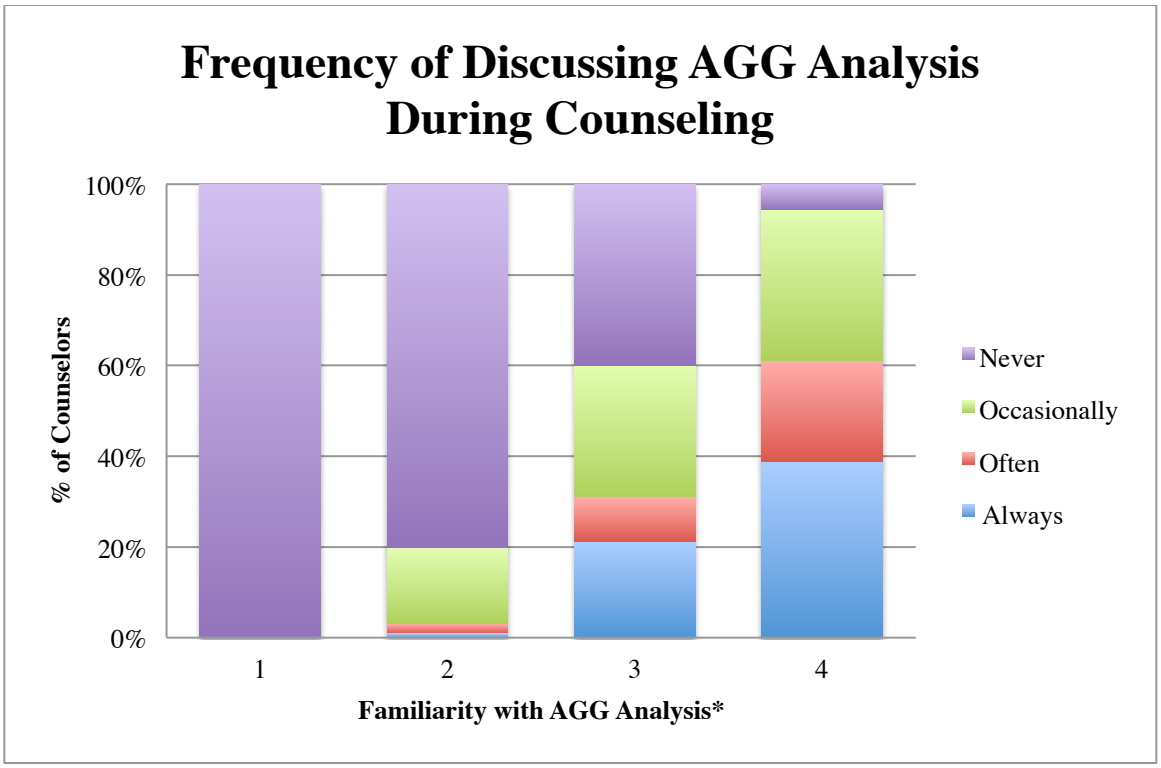


**Figure 7.** NSGC—Frequency of Discussing AGG Analysis Based on Familiarity

*Respondents (n=256) previously rated their familiarity with AGG analysis from not at all knowledgeable (1) to very knowledgeable (4), as can be seen in Figure 4.

**Table 3.** NSGC—Frequency of Discussing AGG Analysis Based on Familiarity

| When counseling patients with an FMR1 intermediate or premutation allele, how often do you discuss the role of AGG analysis to refine the risk of expansion in the FMR1 gene? | | |
|---|---|---|
| | Never | Occasionally, Often or Always |
| Knowledge Level 1 or 2 | 111 | 19 |
| Knowledge Level 3 or 4 | 38 | 88 |

One-tailed Fisher's exact test: **p-value = <0.0001**

A large percentage of counselors (79.3%; n=284) have never offered AGG testing to patients. For those who have offered AGG testing (20.7%; n=74), most cited reasons such as assisting pregnant patients in making decisions regarding prenatal diagnosis and/or assisting patients in making decisions about family planning. Only one counselor reported offering testing "as part of a research study", while a small handful mentioned "standard of care" or by "patient request", as can be seen in Figure 8.

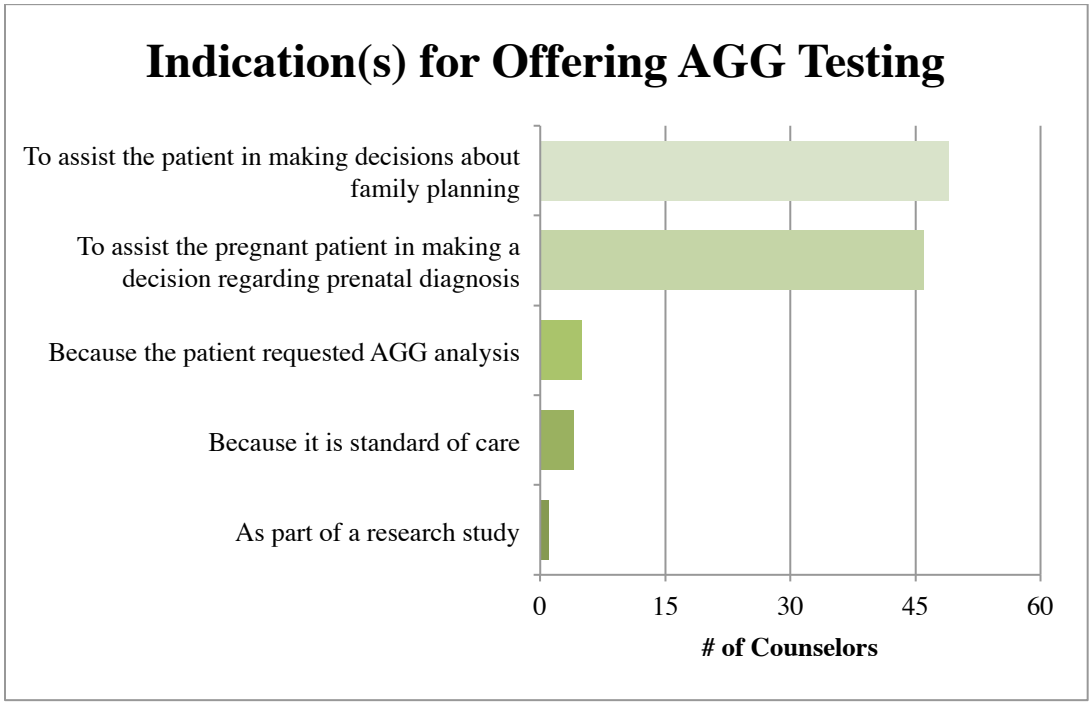


**Figure 8.** NSGC—Indications for Offering AGG Testing

*Respondents (n=74) were able to select multiple indications.

In order to extrapolate patient demand, counselors were asked if patients had ever asked about additional testing (such as AGG analysis) to help refine their risk for expansion in the *FMR1* gene. Out of the 383 respondents, only 21 counselors (5.48%) reported patients asking about their additional testing options. Not surprisingly, these 21 counselors reported seeing a

greater number of patients than other counselors taking this survey. Polling these 21 individuals, they each counseled ~18.6 patients with an *FMR1* intermediate or premutation allele over the past 3 years (SD ± 24.0; range 0 to 100). This is compared to an average of ~6.4 patients for all survey respondents.

### 3.1.4 Barriers to Offering/Ordering AGG Testing

Counselors were asked to answer a series of true/false questions in order to assess their perceived barriers (if any) to offering/ordering AGG testing (total n=350). While most counselors (90.9%; n=318) felt testing was relevant for their patients with an *FMR1* intermediate or premutation allele, a majority (67.7%; n=237) desired more education before they would feel knowledgeable enough to discuss this with their patients. An equally large percentage of counselors (66.3%; n=232) were concerned about insurance coverage and/or the additional cost this testing would add for their patient. A smaller proportion of counselors (40.3%; n=141) reported either not knowing this testing was available or not knowing how to go about requesting it. Finally, one-third of counselors (33.1%; n=116) felt information gained from AGG analysis added further complexity to *FMR1* genetic counseling without actually changing patient decision making, as can be seen in Figure 9.

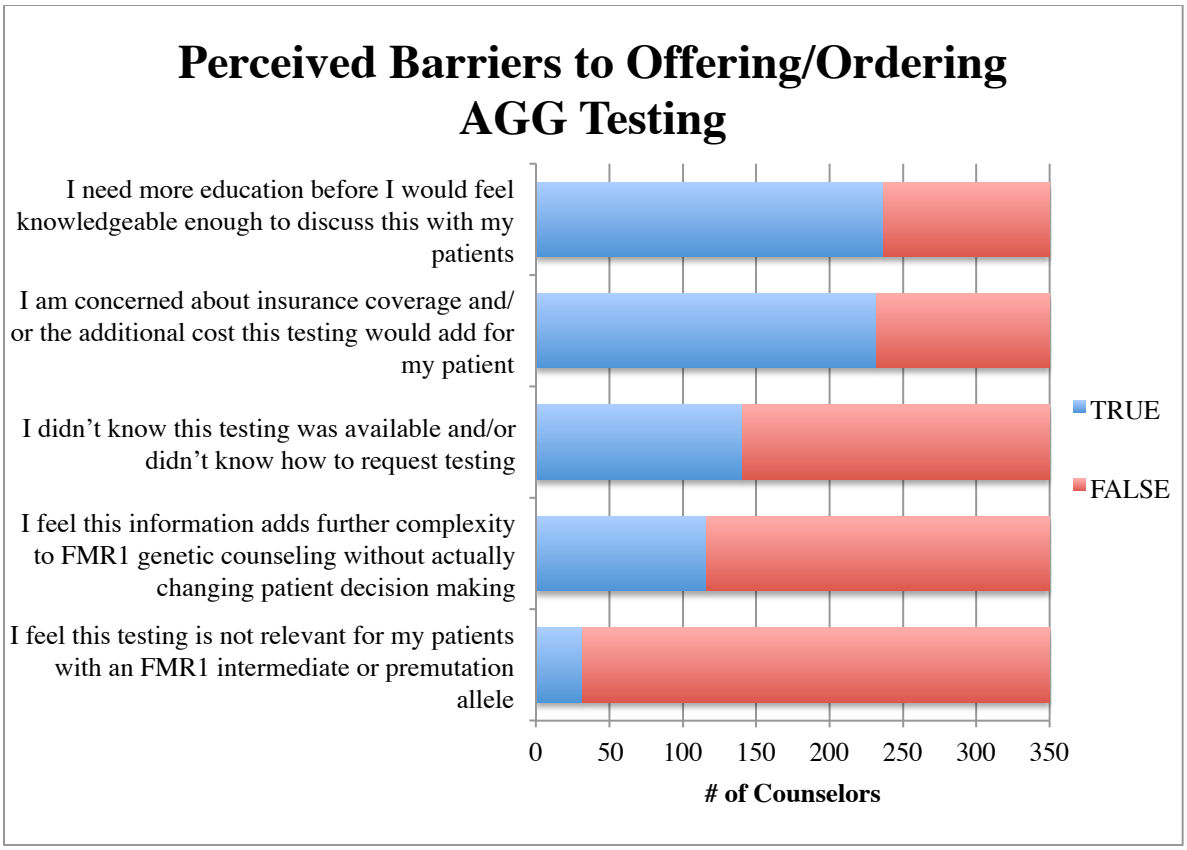


**Figure 9.** NSGC—Perceived Barriers to Offering/Ordering AGG Testing

The above question was further stratified to determine the impact of AGG knowledge on each of the perceived barriers. As anticipated, counselors who considered themselves less knowledgeable about AGG analysis were more likely to select "I need more education before I would feel knowledgeable enough to discuss this with my patients" and "I didn't know this testing was available and/or didn't know how to request testing", as can be seen in Figure 10 and Tables 4-5. Counselors' level of knowledge was not a significant predictor for concerns regarding insurance, complexity, or irrelevance, as can be seen in Figure 11 and in Appendix E, Tables 16-18.

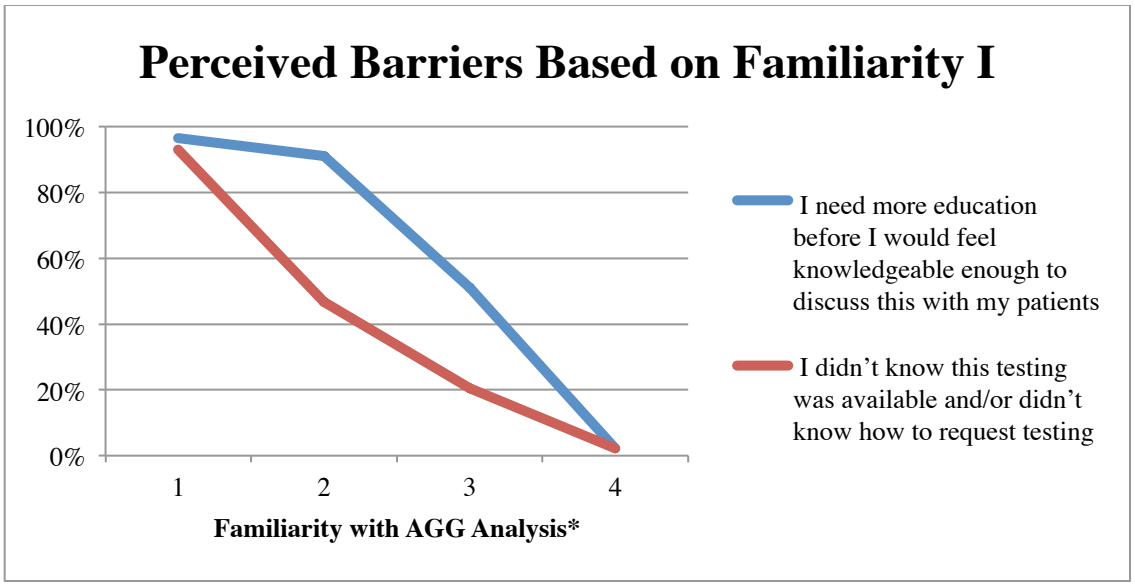


**Figure 10.** NSGC—Perceived Barriers Based on Familiarity I

*Respondents (n=350) previously rated their familiarity with AGG analysis from not at all knowledgeable (1) to very knowledgeable (4), as can be seen in Figure 4.

**Table 4.** NSGC—Perceived Barriers Based on Familiarity I: Education

| I need more education before I would feel knowledgeable enough to discuss this with my patients | | |
|---|---|---|
| | TRUE | FALSE |
| Knowledge Level 1 or 2 | 179 | 14 |
| Knowledge Level 3 or 4 | 58 | 99 |

One-tailed Fisher's exact test: **p-value = <0.0001**

**Table 5.** NSGC—Perceived Barriers Based on Familiarity I: Awareness

| I didn't know this testing was available and/or didn't know how to request testing | | |
|---|---|---|
| | TRUE | FALSE |
| Knowledge Level 1 or 2 | 117 | 76 |
| Knowledge Level 3 or 4 | 24 | 133 |

One-tailed Fisher's exact test: **p-value = <0.0001**

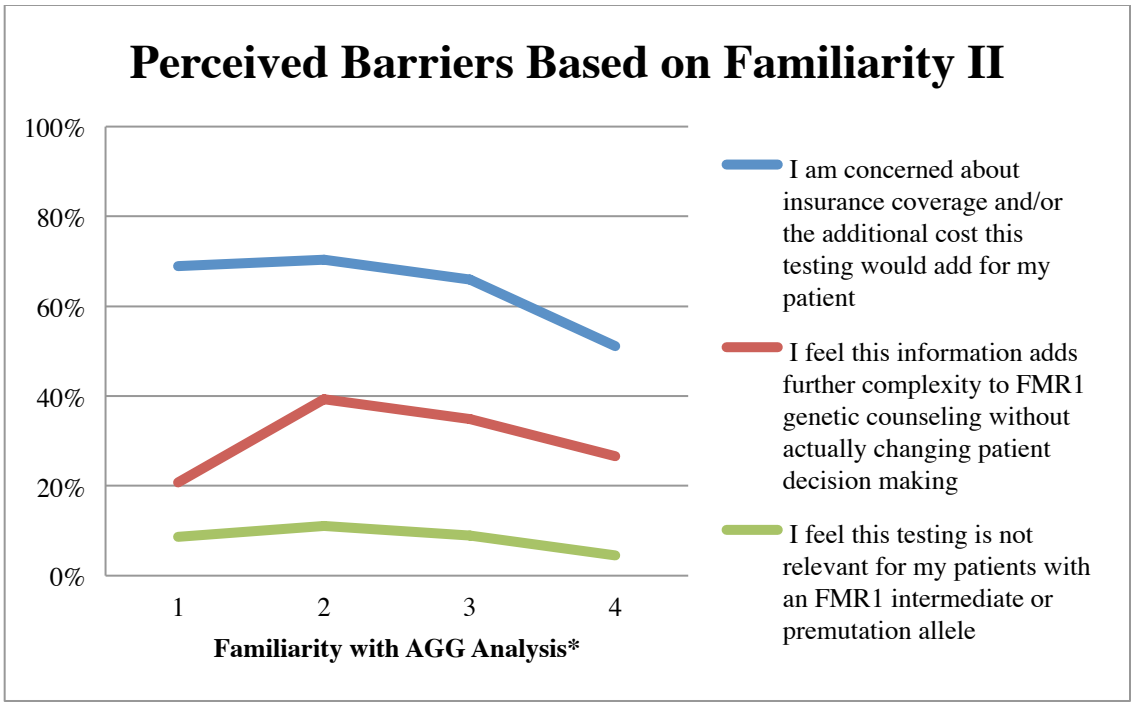


**Figure 11.** NSGC—Perceived Barriers Based on Familiarity II

*Respondents (n=350) previously rated their familiarity with AGG analysis from not at all knowledgeable (1) to very knowledgeable (4), as can be seen in Figure 4.

Perceived barriers to offering/ordering AGG testing were again stratified to determine if perceptions differed among those who have offered AGG testing in the past as compared to those who have not. As anticipated, counselors who have offered testing in the past were less likely to select "I need more education before I would feel knowledgeable enough to discuss this with my patients" and "I didn't know this testing was available and/or didn't know how to request testing", as can be seen in Tables 6-7. Counselors past use of AGG testing was not a significant predictor for concerns regarding insurance, complexity, or irrelevance, as can be seen in Appendix E, Tables 19-21.

**Table 6.** NSGC—Perceived Barriers Based on Past Use: Education

| I need more education before I would feel knowledgeable enough to discuss this with my patients | | |
|---|---|---|
| | TRUE | FALSE |
| Have offered AGG analysis to patients in the past | 8 | 60 |
| Have NOT offered AGG analysis to patients in the past | 229 | 53 |

One-tailed Fisher's exact test: **p-value = <0.0001**

**Table 7.** NSGC—Perceived Barriers Based on Past Use: Awareness

| I didn't know this testing was available and/or didn't know how to request testing | | |
|---|---|---|
| | TRUE | FALSE |
| Have offered AGG analysis to patients in the past | 1 | 67 |
| Have NOT offered AGG analysis to patients in the past | 140 | 142 |

One-tailed Fisher's exact test: **p-value = <0.0001**

### 3.1.5 Future Use of AGG Analysis

The majority of counselors (76.8%; n=275) reported being "more likely" to utilize AGG analysis in the presence of a family history consistent with possible Fragile X syndrome, Fragile X-associated Tremor/Ataxia syndrome, or Fragile X-associated Primary Ovarian Insufficiency. Conversely, the remainder of counselors (23.2%; n=83) said they were "less likely" to utilize this testing methodology in the presence of those family histories.

Counselors were further asked if there were certain CGG repeat ranges that would make them more likely to discuss the option of AGG testing in the future. Respondents were able to mark all repeat ranges that applied. Sixty-seven counselors (18.8%; total n=357) did not plan to discuss this testing with their patients. Of the remaining 290 counselors who answered with

specific repeat ranges, about half (45.9%; n=133) would discuss AGG testing with someone with an intermediate allele (45-54 CGG repeats). Furthermore, over 65% of counselors would discuss AGG testing with a premutation allele carrier with 55-69 CGG repeats. In addition, the majority of counselors (59.7%; n=173) would discuss this testing option with individuals with 70 or higher CGG repeats, as can be seen in Figure 12. Interestingly, there were 55 counselors (20.0%) who selected all CGG repeat ranges (from an intermediate allele with 45 CGG repeats up to a premutation allele with 70 or more CGG repeats). This could be implying they would discuss AGG testing with an *FMR1* carrier regardless of repeat length. Counselors with greater knowledge of AGG analysis were significantly more likely to discuss this additional testing with a premutation allele carrier with 60-69 CGG repeats, as can be seen in Tables 8-9. Knowledge did not seem to influence the likelihood of discussion for other repeat ranges, as can be seen in Appendix E, Tables 22-24.

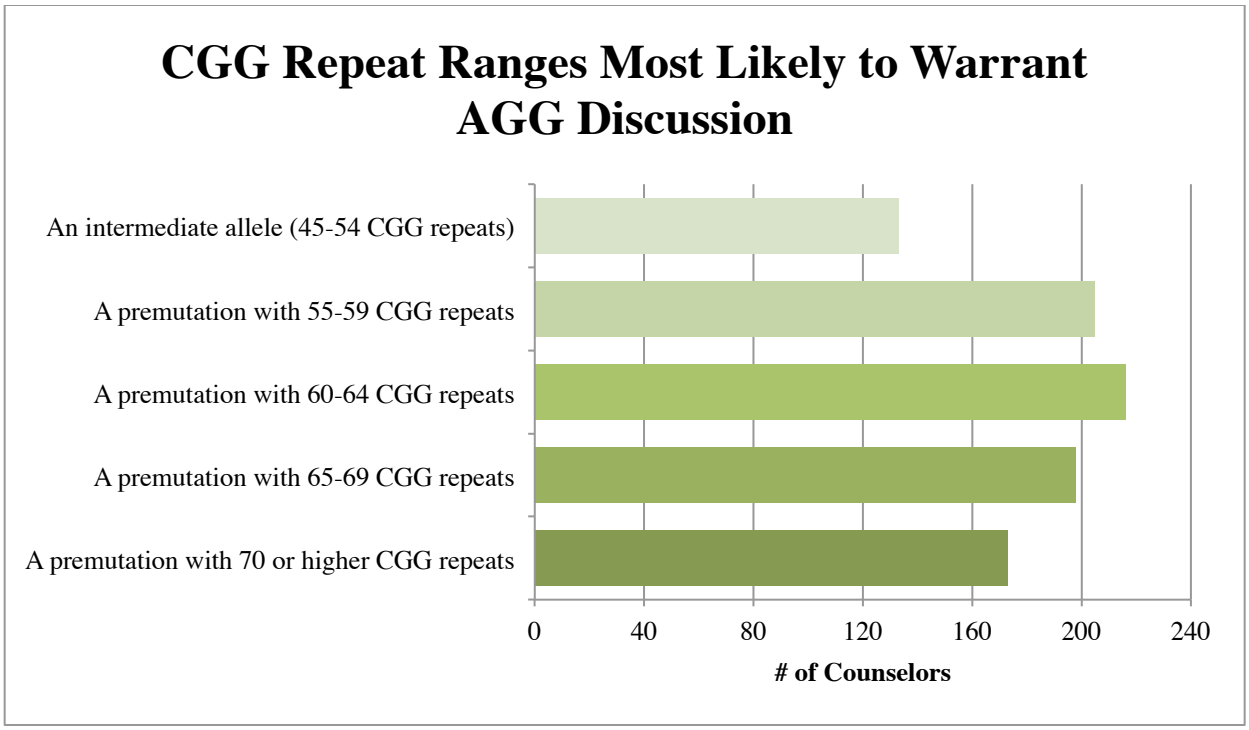


**Figure 12.** NSGC—CGG Repeat Ranges Most Likely to Warrant AGG Discussion

*Respondents (n=290) were able to select all repeat ranges that applied. Counselors not planning to discuss this testing with their patients were excluded from this analysis.

**Table 8.** NSGC—AGG Discussion Based on Familiarity: 60-64 repeats

| A premutation with 60-64 CGG repeats | | |
|---|---|---|
| | Yes | No |
| Knowledge Level 1 or 2 | 95 | 51 |
| Knowledge Level 3 or 4 | 122 | 23 |

Two-tailed Fisher's exact test: **p-value = 0.0002**

**Table 9.** NSGC—AGG Discussion Based on Familiarity: 65-69 repeats

| A premutation with 65-69 CGG repeats | | |
|---|---|---|
| | Yes | No |
| Knowledge Level 1 or 2 | 87 | 59 |
| Knowledge Level 3 or 4 | 112 | 33 |

Two-tailed Fisher's exact test: **p-value = 0.002**

Another portion of the survey was designed to capture whether or not counselors planned to use AGG analysis differently in the future. While only 19.0% (n=68) of respondents report offering AGG analysis to patients in the past, the vast majority of counselors surveyed (86.9%; n=311) say they would consider offering AGG analysis in the future. Interestingly, one individual who reported offering the testing in the past does not plan to offer this testing again in the future. Furthermore, another 12.9% (n=46) have not offered AGG analysis to patients in the past and do not plan to offer this testing in the future. This breakdown can be seen in Table 10.

**Table 10.** NSGC—Counselors Past and Future Use of AGG Analysis

| Counselors Past and Future Use of AGG Analysis | | |
|---|---|---|
| | Would consider offering in the future | Do NOT plan to offer this testing in the future |
| Have offered AGG analysis to patients in the past | 68 | 1 |
| Have NOT offered AGG analysis to patients in the past | 243 | 46 |

Two-tailed Fisher's exact test: **p-value = 0.001**

### 3.1.6 Establishment of Formal Guidelines

There was a divide amongst genetic counselors participating in this survey, with just under half (44.5%; n=155) believing that informing patients about the option of AGG analysis should be the standard of care for genetics service providers. Counselors rating themselves with a higher level of knowledge about AGG analysis were more likely to believe informing patients should be the standard of care, as can be seen in Table 11.

**Table 11.** NSGC—Standard of Care

| Should informing patients about the option of AGG analysis be the standard of care for genetics service providers? | | |
|---|---|---|
| | Yes | No |
| Knowledge Level 1 or 2 | 67 | 124 |
| Knowledge Level 3 or 4 | 88 | 69 |

Two-tailed Fisher's exact test: **p-value = <0.0001**

A strong majority of counselors (90.2%; n=314) felt formal guidelines should be established for the purpose of identifying who should be offered AGG analysis. Amongst these individuals, many believed either the National Society of Genetic Counselors or the American College of Medical Genetics should be the organization responsible for establishing these guidelines. A more detailed breakdown of organizational preferences can be seen in Figure 13.

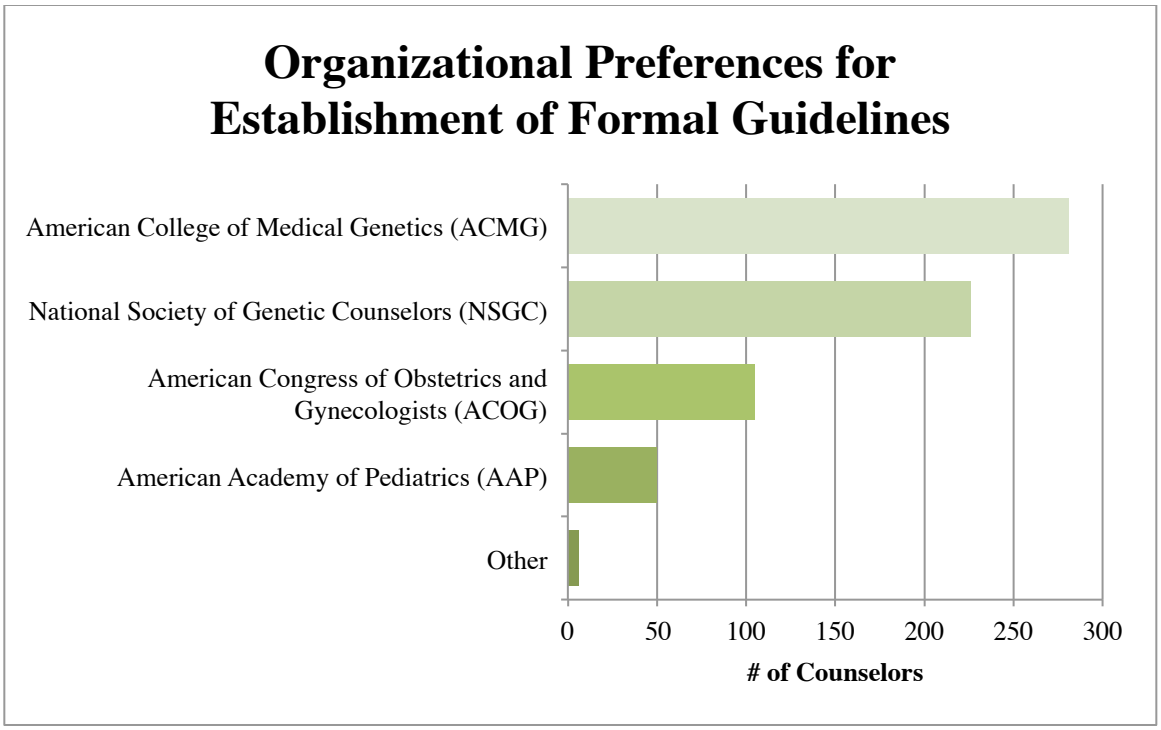


**Figure 13.** NSGC—Organizational Preferences for Establishment of Formal Guidelines

*Respondents (n=313) were able to select multiple organizations.

## 3.2 INTERNATIONAL RESPONDENT STATISTICS

### 3.2.1 Demographics

Two hundred and seventy-one ASGC members and approximately three hundred members of AGNC were e-mailed to participate in this research study. Of these, sixteen ASGC members (6%) and twelve AGNC members (4%) took part in the survey. Of the AGNC respondents, one was a genetic nurse and the rest were genetic counselors. The majority of respondents (64.3%; n=18) had been practicing for five or more years. The mean years of practice was 8.09 years (SD ± 7.04; range 0.5 to 30 years; n=28). The median was 7 years of practice.

The survey further elicited which specialty area(s) counselors practiced in. A majority (67.9%; n=19) of survey respondents reported specializing in prenatal genetics. Many of these individuals reported specializing in additional areas of genetics as well. Interestingly, the bulk of counselors reported specializing in adult genetics (78.6%; n=22). Figure 14 shows the distribution of specialty area(s) respondents. In the "Other" category, respondents reported specializing in areas such as X-linked disorders, pre-implantation genetic diagnosis, and learning disabilities. Of the seven counselors who marked "Other", most of them (71.4%; n=5) also identified with at least one of the provided answer choices.

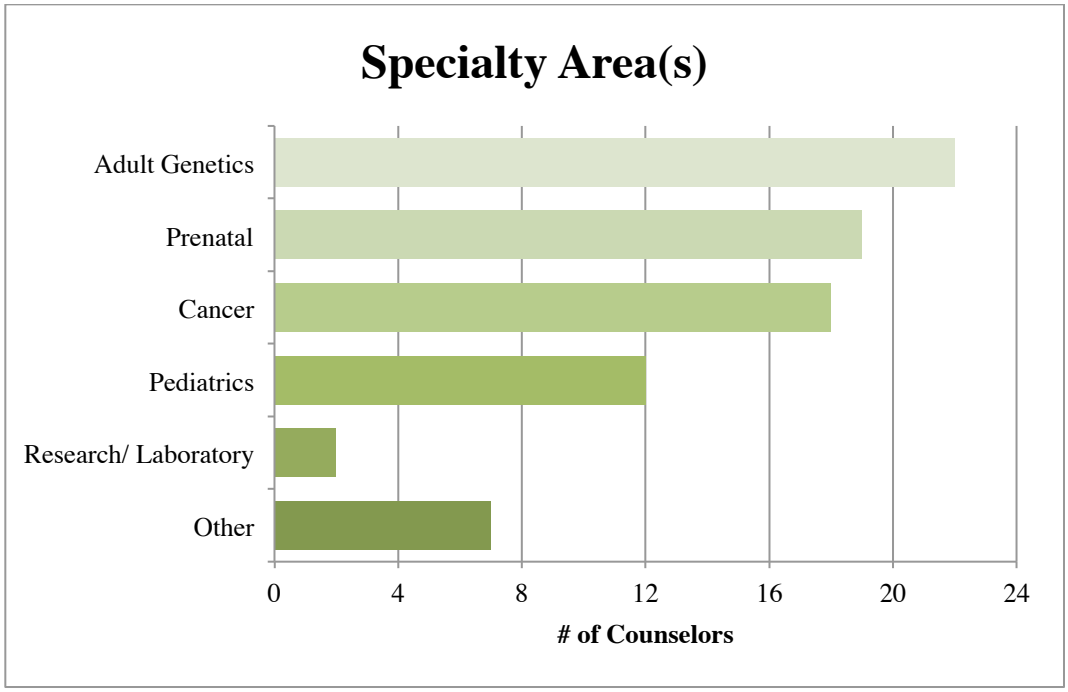


**Figure 14.** International—Specialty Area(s)

*Respondents (n=28) were able to select multiple specialty areas.

### 3.2.2 Knowledge of AGG Analysis

In order to assess knowledge levels, international counselors (n=27) were asked how familiar they were with the notion of analyzing AGG interruptions in women with an *FMR1* intermediate or premutation allele. The counselors rated themselves on a scale of 1 to 4, with 1 being not at all knowledgeable and 4 being very knowledgeable. Only 11.1% of counselors (n=3) considered themselves very knowledgeable, whereas most counselors (74.1%; n=20) reported themselves as not at all knowledgeable, as can be seen in Figure 15. International respondents were less knowledgeable about AGG analysis than NSGC respondents, as can be seen in Table 12. Amongst the counselors with at least some degree of knowledge, education about AGG analysis was obtained by reading peer-reviewed articles on the studies performed to date, attending lectures/presentations on AGG analysis and/or discussing it with either a company that performs the testing or their own local laboratory.

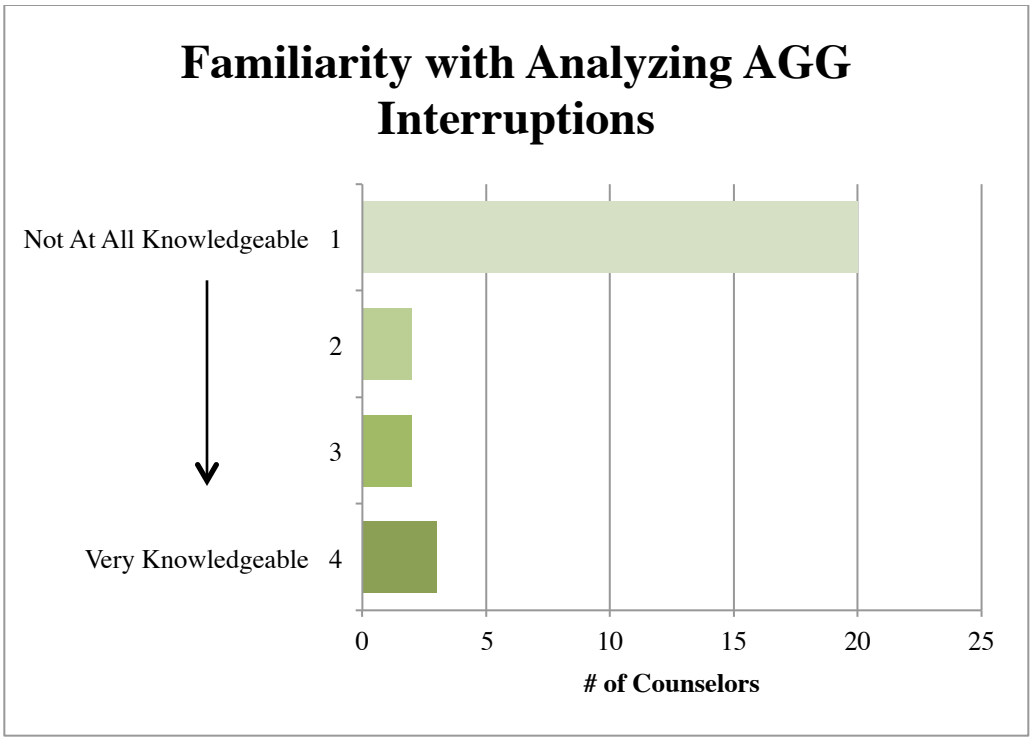


**Figure 15.** International—Familiarity with Analyzing AGG Interruptions

*Respondents (n=27) rated their familiarity from not at all knowledgeable (1) to very knowledgeable (4).

**Table 12.** Differing Familiarity for International vs. NSGC Respondents

| Familiarity with Analyzing AGG Interruptions | | |
|---|---|---|
| | International | NSGC |
| Knowledge Level 1 or 2 | 22 | 221 |
| Knowledge Level 3 or 4 | 5 | 165 |

One-tailed Fisher's exact test: **p-value = 0.009**

### 3.2.3 FMR1 Patient Experience

Of the 28 international respondents, all reported that they counsel patients. Further, the majority of counselors taking part in this survey (89.3%; n=25) have counseled a patient with an *FMR1* intermediate or premutation allele. Polling these 25 individuals, they have on average each counseled ~5.7 patients with an *FMR1* intermediate or premutation allele over the past 3 years (SD ± 6.3; range 0 to 20). The median number of patients counseled with an *FMR1* intermediate or premutation allele over the past 3 years was 3. The distribution of *FMR1* patients counseled over the past 3 years is shown in Figure 16.

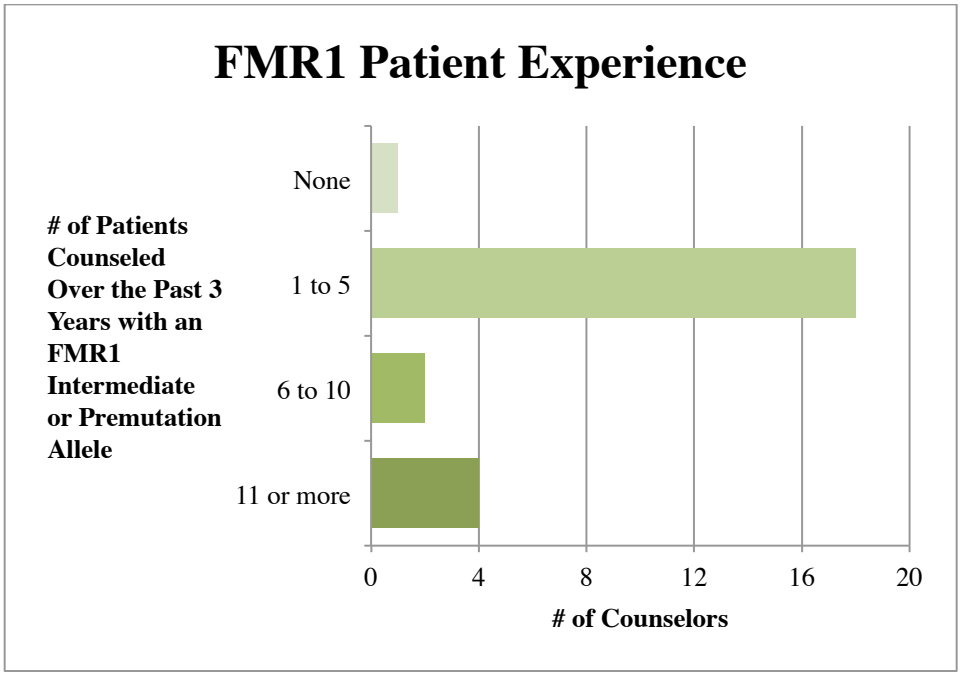


**Figure 16.** International—*FMR1* Patient Experience

Counselors were asked how often they discuss the role of AGG analysis when counseling patients with an *FMR1* mutation. Two respondents felt this question was not applicable to them,

likely because they do not counsel patients for this indication. Of the remaining 21 counselors, the majority (81.0%; n=17) "never" discussed the role of AGG analysis. Very few (19.0%; n=4) report discussing the role of AGG analysis "occasionally" or "often", as can be seen in Figure 17. No one selected "always". Regardless of whether or not counselors chose to discuss AGG analysis in session, none of them (n=23) had offered clinical AGG testing and only two counselors (8.7%) had offered AGG testing as part of a research study.

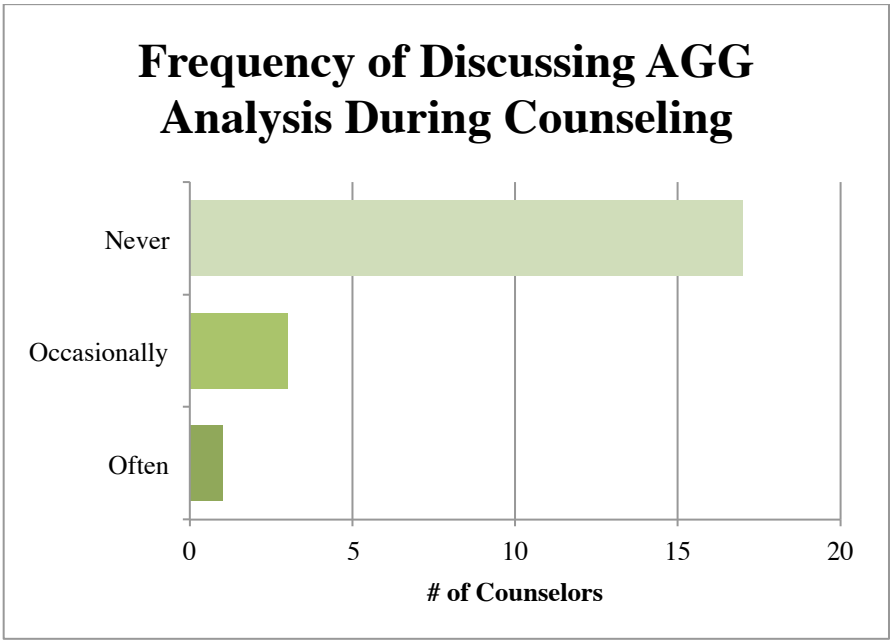


**Figure 17.** International—Frequency of Discussing AGG Analysis During Counseling

In order to extrapolate patient demand, counselors were asked if patients had ever asked about additional testing (such as AGG analysis) to help refine their risk for expansion in the *FMR1* gene. Out of the 27 respondents, only 1 counselor (3.7%) reported patients asking about their additional testing options.

### 3.2.4 Barriers to Offering/Ordering AGG Testing

International counselors were asked to answer a series of true/false questions in order to assess their perceived barriers (if any) to offering/ordering AGG testing (total n=23). The majority of counselors (87.0%; n=20) either didn't know this testing was available or didn't know how to request it. In addition, the majority of respondents (82.6%; n=19) desired more education before they would feel knowledgeable enough to discuss this with their patients. Most counselors did not report insurance concerns, complexity, or irrelevance as obstacles preventing them from offering/ordering AGG testing, as can be seen in Figure 18.

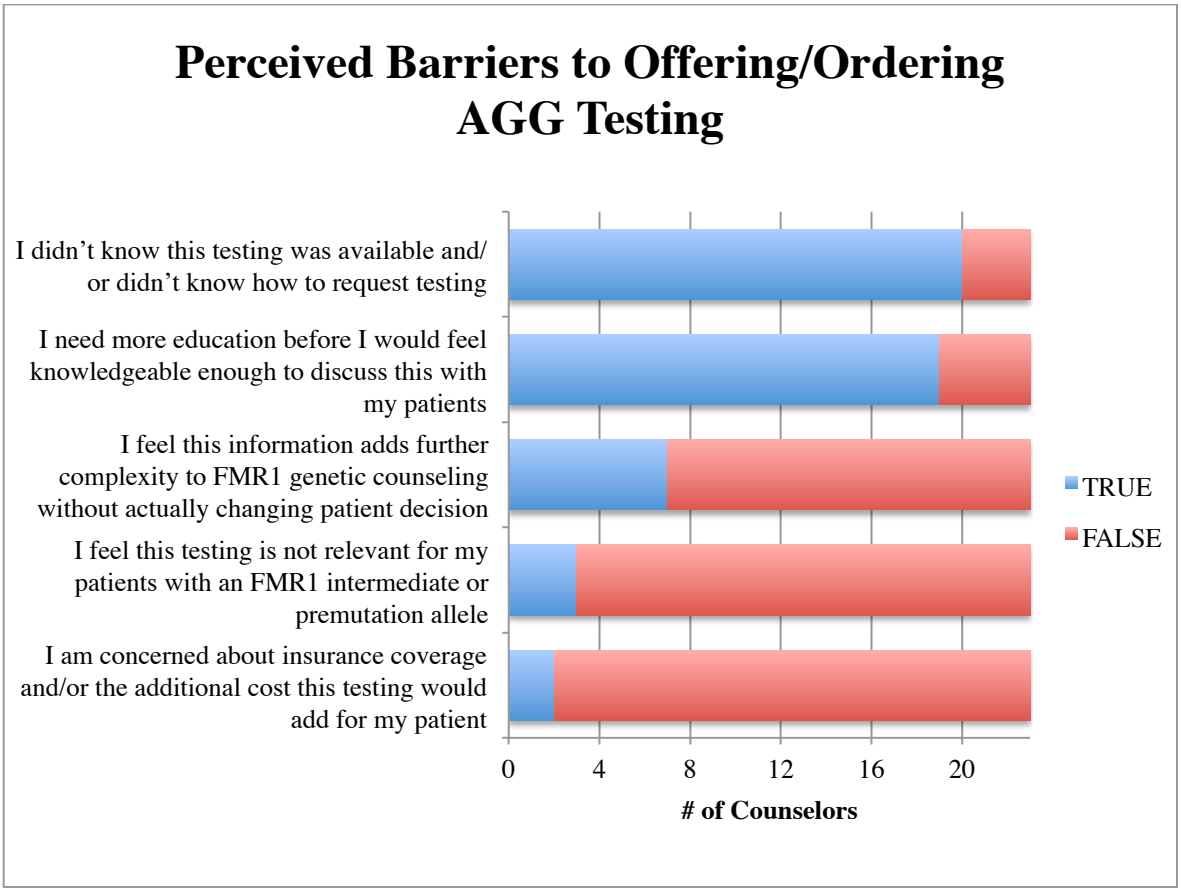


**Figure 18.** International—Perceived Barriers to Offering/Ordering AGG Testing

Perceived barriers to offering/ordering AGG testing were stratified to determine if perceptions differed among international versus NSGC respondents. International counselors were significantly more likely to select "I didn't know this testing was available and/or didn't know how to request testing", as can be seen in Table 13. In addition, international counselors were statistically less likely to mark down concerns about insurance coverage, as can be seen in Table 14. Counselors need for education, concerns about complexity and feelings of irrelevance were not significantly influenced by affiliation with the NSGC or an international organization, as can be seen in Appendix E, Tables 25-27.

**Table 13.** Perceived Barriers for International vs. NSGC Respondents: Awareness

| I didn't know this testing was available and/or didn't know how to request testing | | |
|---|---|---|
| | TRUE | FALSE |
| International | 20 | 3 |
| NSGC | 141 | 209 |

One-tailed Fisher's exact test: **p-value = <0.0001**

**Table 14.** Perceived Barriers for International vs. NSGC Respondents: Insurance

| I am concerned about insurance coverage and/or the additional cost this testing would add for my patient | | |
|---|---|---|
| | TRUE | FALSE |
| International | 2 | 21 |
| NSGC | 232 | 118 |

Two-tailed Fisher's exact test: **p-value = <0.0001**

### 3.2.5 Future Use of AGG Analysis

The majority of counselors (73.9%; n=17) reported being "more likely" to utilize AGG analysis in the presence of family history consistent with possible Fragile X syndrome, Fragile X-associated Tremor/Ataxia syndrome, or Fragile X-associated Primary Ovarian Insufficiency. Conversely, the remainder of counselors (26.1%; n=6) said they were "less likely" to utilize this testing methodology in the presence of those family histories.

International respondents were further asked if there were certain CGG repeat ranges that would make them more likely to discuss the option of AGG testing in the future. However, this data was not analyzed because the majority of international respondents had previously rated themselves as not at all knowledgeable about analyzing AGG interruptions. Therefore the given answers were largely guesses.

Another portion of the survey was designed to capture whether or not counselors planned to use AGG analysis differently in the future. The majority of counselors surveyed (91.3%; n=21) say they would consider offering AGG analysis in the future. Two counselors (2.9%; n=46) who have not offered AGG analysis to patients in the past shared they do not plan to offer this testing in the future. This breakdown can be seen in Table 15.

**Table 15.** International—Counselors Past and Future Use of AGG Analysis

| Counselors Past and Future Use of AGG Analysis | | |
|---|---|---|
| | Would consider offering in the future | Do NOT plan to offer this testing in the future |
| Have offered AGG analysis to patients in the past | 1 | 0 |
| Have NOT offered AGG analysis to patients in the past | 20 | 2 |

### 3.2.6 Establishment of Formal Guidelines

The majority of international counselors (72.7%; n=16) did not believe informing patients about the option of AGG analysis should be the standard of care for genetics service providers. This approached but did not reach statistical significance as compared to NSGC respondents (Appendix E, Table 28). However, a strong majority (90.9%; n=20) felt formal guidelines should be established for the purpose of identifying who should be offered AGG analysis. While some respondents thought either the American College of Medical Genetics or the National Society of Genetic Counselors should be the organization responsible for establishing these guidelines, others specified organizations such as the Human Genetics Society of Australasia, the British Society for Genetic Medicine, and the Association of Genetic Nurses and Counsellors. A more detailed breakdown of organizational preferences can be seen in Figure 19.

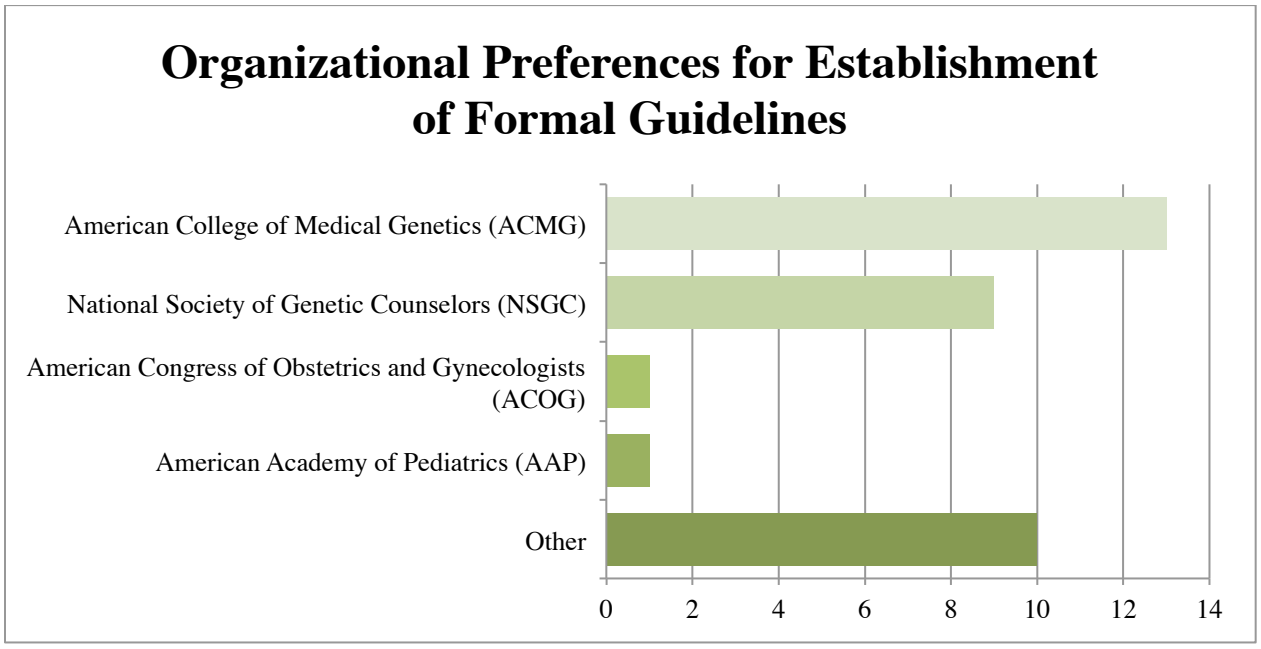


**Figure 19.** International—Organizational Preferences for Establishment of Formal Guidelines

*Respondents (n=20) were able to select multiple organizations.

# 4.0 DISCUSSION

This research was an effort to better understand genetic counselors' knowledge, utilization, and attitudes towards the clinical utility of AGG analysis for their patients. The research further served to assess the desire for formal testing guidelines. Information gathered from this study will be of benefit to the genetic counseling profession and may impact the utilization of genetic testing methodologies in the future.

## 4.1 DISCUSSION OF NSGC RESULTS

### 4.1.1 Demographics

Of the two thousand nine hundred and thirty-two NSGC members invited to participate in this survey, three hundred and ninety members (13%) agreed to participate. Almost all (95%) reported working primarily in the United States. The rest worked in Canada and one counselor reported working in Australia. Experience ranged from 0 to 39 years, with an average of 8 years. Approximately half of the counselors surveyed had been practicing for five years or less.

The majority (60%) of survey respondents reported specializing in prenatal genetics. This can be compared to the 2012 NSGC Professional Status Survey, which documented 29% of genetic counselors in prenatal genetics. It would seem that genetic counselors practicing in this

specialty were more likely to take an interest in this survey. Pediatric genetics was the next most common specialty area. Not surprisingly, a few individuals mentioned their involvement in infertility, which has been a growing area for Fragile X carrier testing.

### 4.1.2 Knowledge of AGG Analysis

Most counselors (82%) reported at least some degree of knowledge about AGG analysis. However, only 12% of counselors considered themselves very knowledgeable. Amongst counselors with at least some knowledge of AGG analysis, education was obtained in a wide array of methods. Almost half of these counselors reported reading peer-reviewed articles on the studies performed to date. Many also reported attending lectures/presentations on AGG analysis and/or reading promotional materials from a company or institution that performs the testing. A minority of counselors either discussed this testing with a company or institution that performs it, or viewed or participated in a webinar. A few individuals added additional comments citing discussions with colleagues, reading GeneReviews™, in genetic counseling training programs, and while attending NSGC's Annual Education Conference.

### 4.1.3 FMR1 Patient Experience

Almost all the genetic counselors surveyed counsel patients (92%). This can be compared to the 2012 NSGC Professional Status Survey, which documented 77% of respondents counseling patients as a regular part of their jobs. It would seem that those counseling patients were more likely to take an interest in this survey. Further, the majority (79%) have counseled a patient with an *FMR1* intermediate or premutation allele. Polling these individuals, they have on average each counseled ~6.4 patients with an *FMR1* intermediate or premutation allele over the

past 3 years, ranging from 0 to “more than I can count, several 100 maybe more”. The median number of patients counseled with an *FMR1* intermediate or premutation allele over the past 3 years was 3.

Counselors were further asked how often they discuss the role of AGG analysis when counseling patients with an *FMR1* intermediate or premutation allele. About a quarter of the respondents felt this question was not applicable to them, likely because they do not counsel patients for this indication. Of the remaining counselors, the majority (58%) “never” discussed the role of AGG analysis, while only 13% reported “always” discussing AGG analysis. Most interesting was that counselors who considered themselves to be more knowledgeable about AGG analysis were significantly more likely to discuss this topic during counseling. This lends insight to the fact that genetic counselors who were knowledgeable about this testing option perceived it to be important to care. One counselor who ran a Fragile X family support group for almost a decade, commented in the survey that if he/she was still working with patients, he/she would want to “make all patients with an intermediate allele or higher aware of this testing”.

A minority of counselors (21%) have actually offered AGG testing to their patients. For these individuals, most cited reasons such as assisting pregnant patients in making decisions regarding prenatal diagnosis and/or assisting patients in making decisions about family planning. Only one counselor reported offering testing “as part of a research study” and a small handful mentioned “standard of care” or by “patient request”. Some counselors left comments such as, “My patients have liked the opportunity to get a clear risk about the likelihood of having a future child with Fragile X” and “AGG analysis often plays a role in a patient's decision to undergo invasive testing. It has the potential to clarify the risk for expansion in pregnancies and future pregnancies. My patients have been thankful for the additional information”. Another counselor

shared how AGG results had significantly altered their patient's choices about pre-implantation genetic diagnosis.

In order to extrapolate patient demand, counselors were asked if patients had ever asked about additional testing (such as AGG analysis) to help refine their risk for expansion in the *FMR1* gene. Only 5% of counselors reported patients asking about their additional testing options. Not surprisingly, those counselors reported seeing almost three times as many patients as other counselors taking this survey.

### 4.1.4 Barriers to Offering/Ordering AGG Testing

Going into this study, the investigators were under the impression counselors were not commonly integrating AGG analysis into their clinical practice conceivably due to limited knowledge, lack of perceived importance in care, and concern for cost burden to patients. As anticipated, the majority of counselors (68%) desired more education before they would feel knowledgeable enough to discuss this test with their patients. This is compounded by 40% of counselors who reported either not knowing this testing was available or not knowing how to go about requesting it.

Two-thirds of counselors were concerned about insurance coverage and/or the additional cost this testing would add for their patient. Furthermore, although not specifically addressed in the survey, a likely inhibitor of testing utilization was laboratory contracts limiting access to specific laboratories. As an example, in the additional comments field of the survey, one counselor explained his/her struggle to convince a laboratory administrator to allow the test to be sent to the appropriate testing facility. In this particular situation, the institution anticipated very little Medicaid reimbursement and the counselor had difficulty justifying how results would

influence the patient's decision making. Another counselor went on to say, "In cases where I have offered [AGG analysis] to patients, I have felt that it will alter the management of their current/future pregnancy… If a patient isn't interested in prenatal diagnosis regardless, and is aware of the risks, I wouldn't see AGG testing as being helpful for management". Some of the Canadian NSGC members shared comments, such as "Government guidelines require that we use local labs before sending away to further labs unless there is a clear advantage to testing in other lab" and "Working in a publically funded healthcare system, I believe we have to think carefully and be very responsible when it comes to deciding which tests are most useful. You shouldn't just offer a test because it is available!"

Surprisingly to the investigators, almost all counselors (91%) felt this testing was relevant for their patients with an *FMR1* intermediate or premutation allele. However, one-third of counselors felt information gained from AGG analysis added further complexity to *FMR1* genetic counseling without actually changing patient decision making. A few counselors confirmed these sentiments in the comments section, making statements like "Most of my population would progress to prenatal diagnosis if they were premutation carriers, regardless of AGG analysis. I'm sure if I worked with a population that was less inclined to have CVS or amnio this answer would be different." Another counselor said in regards to prenatal diagnosis, "I don't think these test results are going to change their choice. Whether the risk for Fragile X is 20%, 2%, or 0.5%, I think they would still choose to test and find out for sure." Taking the opposite stance, one counselor commented that she has a Fragile X premutation allele and would want AGG analysis offered to her. She went on to say that AGG analysis "does increase the complexity of counseling but I feel the patient has a right to know and make the decision for themselves."

Researchers were interested to determine the impact of AGG knowledge on each of the perceived barriers. As anticipated, counselors who considered themselves less knowledgeable about AGG analysis desired more education and/or did not know this testing was available. Counselors' level of knowledge was not a significant predictor for concerns regarding insurance, complexity, or irrelevance.

Researchers further sought to determine if barriers differed among those who have offered AGG testing in the past as compared to those who have not. As expected, counselors who have offered testing in the past were less likely to select "I need more education before I would feel knowledgeable enough to discuss this with my patients" and "I didn't know this testing was available and/or didn't know how to request testing". Counselors past use of AGG testing was not a significant predictor for concerns regarding insurance, complexity, or irrelevance.

### 4.1.5 Future Use of AGG Analysis

Researchers were interested to understand when AGG analysis is most likely to be utilized by genetic counselors. The majority of counselors (77%) reported being "more likely" to utilize AGG analysis in the presence of a family history consistent with possible Fragile X syndrome, Fragile X-associated Tremor/Ataxia syndrome, or Fragile X-associated Primary Ovarian Insufficiency. A few genetic counselors added additional comments to explain that if someone has a family history of Fragile X syndrome, then they would be less likely to utilize AGG analysis because they already know that the allele can expand to a full mutation.

Counselors were further asked if there were certain CGG repeat ranges that would make them more likely to discuss the option of AGG testing in the future. Respondents were able to

mark all repeat ranges that applied. Sixty-seven counselors (19%) did not plan to discuss this testing with their patients. Of the remaining counselors, about half would discuss AGG testing with someone with an intermediate allele (45-54 CGG repeats). While AGG analysis has been marketed as appropriate for known Fragile X carriers with 45-90 repeats, the smallest premutation to expand to a full mutation within a single generation is still 56 repeats (Fernandez-Carvajal et al., 2009).

Interestingly, over 65% of counselors would discuss AGG testing with a premutation allele carrier with 55-69 CGG repeats. In addition, the majority of counselors (60%) would discuss this testing option with individuals with 70 or higher CGG repeats. This makes sense, as AGG interruptions seem to have the greatest influence in risk prediction for alleles with 70-80 CGG repeats. In this repeat range, difference in risk varies by ~60% depending on number of AGGs present (Yrigollen et al., 2012). Finally, 20% of counselors selected all CGG repeat ranges (from an intermediate allele with 45 CGG repeats up to a premutation allele with 70 or more CGG repeats). This is likely implying they would discuss AGG testing with an *FMR1* carrier regardless of repeat length.

Researchers were interested to determine the impact of AGG knowledge on likelihood for discussion based on specific repeat ranges. Counselors with greater knowledge of AGG analysis were significantly more likely to discuss this additional testing with a premutation allele carrier with 60-69 CGG repeats. However, knowledge did not seem to influence the likelihood of discussion for other repeat ranges. One counselor outlined their testing strategy stating, "I have typically offered it to anyone with a CGG repeat between 55 and 70. Of course, if the family history is positive for FX syndrome, I counsel that there is a risk for expansion since it has

obviously expanded in other individuals. For the intermediate range, I offer it but emphasize that expansion to a full mutation has not been identified in a case yet".

Another portion of the survey was designed to capture whether or not counselors planned to use AGG analysis differently in the future. While only 19% of respondents report offering AGG analysis to patients in the past, the vast majority of counselors surveyed (87%) say they would consider offering AGG analysis in the future. This willingness to consider offering AGG analysis in the future, emphasizes that a lack of perceived importance is not a factor hindering genetic counselors in their integration of AGG analysis into clinical practice as previously hypothesized.

### 4.1.6 Establishment of Formal Guidelines

There was a divide amongst genetic counselors participating in this survey, with just under half believing that informing patients about the option of AGG analysis should be the standard of care for genetics service providers. Counselors rating themselves with a higher level of knowledge about AGG analysis were more likely to believe informing patients should be the standard of care. One counselor emphasized in the comments section that their clinic is not typically offering AGG analysis until testing becomes the "gold standard" or a recommendation of a governing organization. This counselor explained that at this point, it has been too difficult to obtain insurance coverage by medical necessity.

At present, there are no formal testing guidelines to direct genetic counselors when to offer AGG analysis to refine risk for expansion. As hypothesized, this study found a high level of desire for the establishment of these guidelines by a governing organization. A strong majority of counselors (90%) felt formal guidelines should be established for the purpose of

identifying who should be offered AGG analysis. Amongst these individuals, many believed either the National Society of Genetic Counselors (NSGC) or the American College of Medical Genetics (ACMG) should be the organization responsible for their establishment. Some counselors also mentioned preferences for the American Congress of Obstetrics and Gynecologists (ACOG) and/or The American Academy of Pediatrics (AAP).

## 4.2 DISCUSSION OF INTERNATIONAL RESULTS

### 4.2.1 Demographics

Two hundred and seventy-one ASGC members and approximately three hundred members of AGNC were invited to participate in this research study. Of these, sixteen ASGC members (6%) and twelve AGNC members (4%) took part in the survey. Of the AGNC respondents, one was a genetic nurse and the rest were genetic counselors. It is problematic to draw any assumptions of the larger organizations based off these small response rates. In this discussion, the researchers hope to shed light on the thoughts and opinions of the survey respondents recognizing that these may be unique to these individuals.

The majority of respondents (64%) had been practicing for five or more years. Experience ranged from 6 months to 30 years, with an average of 8 years. Similar to NSGC respondents, the majority (68%) of counselors reported specializing in prenatal genetics. Many of these individuals reported specializing in additional areas of genetics as well. Unlike the distribution among NSGC respondents, the bulk of international counselors reported specializing in adult genetics (79%). A handful of respondents reported specializing in areas such as X-linked disorders, pre-implantation genetic diagnosis, and learning disabilities.

### 4.2.2 Knowledge of AGG Analysis

Only three counselors (11%) considered themselves very knowledgeable, whereas most counselors (74%) reported themselves as not at all knowledgeable. Overall, international respondents were significantly less knowledgeable about AGG analysis than NSGC respondents. Researchers anticipated this as testing is not clinically available abroad. Amongst the seven counselors with at least some degree of knowledge, education about AGG analysis was obtained by reading peer-reviewed articles on the studies performed to date, attending lectures/presentations on AGG analysis and/or discussing it with either a company that performs the testing or their own local laboratory.

### 4.2.3 FMR1 Patient Experience

All of the international counselors surveyed counsel patients. Further, the majority (89%) have counseled a patient with an *FMR1* intermediate or premutation allele. Polling these individuals, they have on average each counseled ~5.7 patients with an *FMR1* intermediate or premutation allele over the past 3 years, ranging from 0 to 20. The median number of patients counseled with an *FMR1* intermediate or premutation allele over the past 3 years was 3.

Counselors were further asked how often they discuss the role of AGG analysis when counseling patients with an *FMR1* intermediate or premutation allele. Two respondents felt this question was not applicable to them, likely because they do not counsel patients for this indication. Of the remaining counselors, the majority (81%) "never" discussed the role of AGG analysis. Few (19%) report discussing the role of AGG analysis "occasionally" or "often", and no one selected "always". Regardless of whether or not counselors chose to discuss AGG

analysis in session, none of them had offered clinical AGG testing and only two counselors (9%) had offered AGG testing as part of a research study.

In order to extrapolate patient demand, counselors were asked if patients had ever asked about additional testing (such as AGG analysis) to help refine their risk for expansion in the *FMR1* gene. Only 1 counselor (4%) reported patients asking about their additional testing options.

### 4.2.4 Barriers to Offering/Ordering AGG Testing

Going into this study the researchers were not aware of any international counselors that had integrated AGG analysis into their clinical practice. This can likely be largely attributed to the lack of available clinical testing internationally. As projected, the majority of counselors (87%) either didn't know this testing was available or didn't know how to request it. In addition, the majority (83%) desired more education before they would feel knowledgeable enough to discuss this with their patients. Most counselors did not report insurance concerns, complexity, or irrelevance as obstacles preventing them from offering/ordering AGG testing.

Perceived barriers to offering/ordering AGG testing were stratified to determine if perceptions differed among international versus NSGC respondents. International counselors were significantly more likely to select "I didn't know this testing was available and/or didn't know how to request testing". In addition, international counselors were statistically less likely to mark down concerns about insurance coverage. One reason international respondents do not seem to have the same concerns regarding insurance coverage that NSGC respondents do is likely due to the differing healthcare systems in both the UK and Australasia as compared to the United States. Counselors' need for education, concerns about complexity and feelings of

irrelevance were not significantly influenced by affiliation with the NSGC or an international organization.

### 4.2.5 Future Use of AGG Analysis

Researchers were interested to understand when AGG analysis is most likely to be utilized by genetic counselors. The majority of counselors (74%) reported being "more likely" to utilize AGG analysis in the presence of a family history consistent with possible Fragile X syndrome, Fragile X-associated Tremor/Ataxia syndrome, or Fragile X-associated Primary Ovarian Insufficiency.

Another portion of the survey was designed to capture whether or not counselors planned to use AGG analysis differently in the future. Similar to NSGC respondents, the majority of counselors surveyed (91%) said they would consider offering AGG analysis in the future. This willingness to consider offering AGG analysis in the future emphasizes that a lack of perceived importance is not a factor hindering genetic counselors in their integration of AGG analysis into clinical practice as previously hypothesized by this study.

### 4.2.6 Establishment of Formal Guidelines

Unlike NSGC respondents, the majority of international counselors (73%) did not believe informing patients about the option of AGG analysis should be the standard of care for genetics service providers. This approached but did not reach statistical significance. However, this study did find a high level of desire for the establishment of formal testing guidelines by a governing organization. This was true for both NSGC respondents and the majority (91%) of international counselors. While some respondents thought either the American College of

Medical Genetics (ACMG) or the National Society of Genetic Counselors (NSGC) should be the organization responsible for establishing these guidelines, others specified organizations such as the Human Genetics Society of Australasia (HGSA), the British Society for Genetic Medicine (BSGM), and the Association of Genetic Nurses and Counsellors (AGNC).

## 4.3 STUDY LIMITATIONS

This exploratory study aimed to better understand genetic counselors' knowledge, utilization, and attitudes towards the clinical utility of AGG analysis for their patients. Some aspects of the study design must be noted. First, self-selection bias must be acknowledged as participants opted to take part in the survey. While it was not feasible to ascertain reasons for not participating, researchers noticed that those most likely to respond were prenatal genetic counselors and/or those with relevant *FMR1* patient experience. Due to the low response rates, particularly among the international respondents, it is problematic to draw any generalizations of the larger organizations. Rather, these findings should be used to shed light on the thoughts and opinions of the survey respondents recognizing that these may be unique to these individuals. It is possible that participants with stronger viewpoints took part in this survey. Another aspect of the study design that limited interpretation was the requirement to answer each survey question in order to proceed. This was problematic for counselors with little to no knowledge of AGG analysis. At the end of the survey, several counselors commented they wanted to answer "I don't know" or "unsure" but were not given this option. This likely led some counselors to exit the survey before finishing and forced others to provide answers that did not reflect their true responses.

## 4.4 PRACTICE IMPLICATIONS

At present, there are no formal testing guidelines to direct genetic counselors when to offer AGG analysis to refine risk for expansion. This study found a high level of desire for the establishment of these guidelines for the purpose of identifying who should be offered AGG analysis. Many believed either the National Society of Genetic Counselors (NSGC) or the American College of Medical Genetics (ACMG) should be the organization responsible for their establishment. Information gathered from this study will be of benefit to the genetic counseling profession and may impact the utilization of AGG analysis in the future.

## 4.5 RESEARCH RECOMMENDATIONS

Future studies could provide more qualitative analysis through semi-structured interviews. This could include interviewing both genetic counselors who offer and do not offer AGG analysis. Either through individual interviews or focus groups this type of future research could help define what the standard of care will become. Future research is also needed to explore patients' perceptions of the benefits and limitations of AGG analysis in the both the short- and long-term.

# 5.0 CONCLUSION

This research was an effort to better understand genetic counselors' knowledge, utilization, and attitudes towards the clinical utility of AGG analysis for their patients. Almost all survey respondents, in the United States and internationally, reported experience with patients with *FMR1* intermediate or premutation alleles. However, no international respondents and only a minority of NSGC members reported actually offering AGG analysis to their patients. Going into this study, the investigators were under the impression counselors were not commonly integrating AGG analysis into their clinical practice. The hypothesis for this study was that genetic counselors were reserved in their uptake due to limited knowledge, lack of perceived importance in care, and concern for cost burden to patients.

As anticipated, the majority of counselors desired more education before they would feel knowledgeable enough to discuss this test with their patients. This is compounded by the fact that 40% of NSGC counselors, and an even greater percentage of international counselors, reported either not knowing this testing was available or not knowing how to go about requesting it. NSGC counselors were significantly more knowledgeable about AGG analysis as compared to their international counterparts. This is likely due to the absence of the clinical availability of this testing abroad. Counselors who considered themselves to be more knowledgeable about AGG analysis were significantly more likely to discuss this topic with patients during counseling.

Surprisingly to the investigators, almost all counselors felt AGG analysis was relevant for their patients with an *FMR1* intermediate or premutation allele. Researchers had postulated that information gained from AGG analysis added further complexity to *FMR1* genetic counseling without actually changing patient decision making. It was thought that this lack of perceived importance might have influenced the low uptake of this testing methodology. In actuality, only one-third of NSGC counselors felt this way. In addition, knowledge about AGG analysis was not a significant predictor for how counselors would respond to this question.

While only 19% of NSGC respondents report offering AGG analysis to patients in the past, the vast majority of NSGC and international counselors surveyed say they would consider offering AGG analysis in the future. This willingness to consider offering this testing in the future, further emphasizes that a lack of perceived importance is not a factor hindering genetic counselors in their integration of AGG analysis into clinical practice as previously hypothesized. This was reiterated by data showing NSGC counselors with a higher level of AGG knowledge were more likely to believe informing patients should be the standard of care.

As expected by the investigators, two-thirds of NSGC counselors were concerned about insurance coverage and/or the additional cost this testing would add for their patient. International respondents were less likely to report this as a perceived barrier to ordering/offering testing.

At present, there are no formal testing guidelines to direct genetic counselors when to offer AGG analysis to refine risk for expansion. As hypothesized, this study found a high level of desire for the establishment of these guidelines by a governing organization. A strong majority of counselors felt formal guidelines should be established for the purpose of identifying who should be offered AGG analysis. Amongst these individuals, many believed either the

National Society of Genetic Counselors (NSGC) or the American College of Medical Genetics (ACMG) should be the organization responsible for their establishment.

Information gathered from this study will be of benefit to the genetic counseling profession and may impact the utilization of genetic testing methodologies in the future.

## APPENDIX A: IRB EXEMPTION LETTER

**University of Pittsburgh**
***Institutional Review Board***

3500 Fifth Avenue
Pittsburgh, PA 15213
(412) 383-1480
(412) 383-1508 (fax)
http://www.irb.pitt.edu

**Memorandum**

To: Lauren Brown
From: Sue Beers, PhD, Vice Chair
Date: 9/27/2013
IRB#: PRO13090270
Subject: Exploring genetic counselors' perceptions and utilization of AGG analysis to refine FMR1 gene expansion risk

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The above-referenced project has been reviewed by the Institutional Review Board. Based on the information provided, this project meets all the necessary criteria for an exemption, and is hereby designated as "exempt" under section
45 CFR 46.101(b)(2).

The IRB has approved the advertisement that was submitted for review as written. As a reminder, any changes to the approved advertisement would require IRB approval prior to distribution.

Please note the following information:

- If any modifications are made to this project, use the "**Send Comments to IRB Staff**" process from the project workspace to request a review to ensure it continues to meet the exempt category.
- Upon completion of your project, be sure to finalize the project by submitting a " **Study Completed**" report from the project workspace.

**Please be advised that your research study may be audited periodically by the University of Pittsburgh Research Conduct and Compliance Office.**

# APPENDIX B: RECRUITMENT MATERIALS

## B.1 RECRUITMENT BROCHURE

GENETIC COUNSELORS,

Help with Fragile X Research!

Complete a brief, online, anonymous survey. Your insight would be valuable, even if you do not regularly see patients with mutations in their FMR1 gene.

We would appreciate your response by *Friday, November 8, 2013.*

Questions?

Lauren H. Brown, BA
Genetic Counseling Intern
University of Pittsburgh
(360) 606-4706
Lhb11@pitt.edu

**https://www.surveymonkey.com/s/FragileXtesting**

## B.2 RECRUITMENT E-MAILS

### B.2.1 NSGC Recruitment E-mail

Dear *Genetic Counselor*,

I am second year genetic counseling student at the University of Pittsburgh. I am collaborating on a research study, with some of the leaders in the Fragile X field, to explore genetic counselors' perceptions and utilization of additional testing to refine the risk of expansion in the *FMR1* gene.

It is our hope that information gathered from this study will be of benefit to the genetic counseling profession and impact the utilization of genetic testing methodologies in the future. Your insight would be valuable, even if you do not regularly see patients with mutations in their *FMR1* gene.

If you agree to participate, you will be asked to complete a brief, online survey. Completion of the anonymous survey should take no longer than 10 minutes. Simply click on the link below, or cut and paste the entire URL into your browser to access the survey:

https://www.surveymonkey.com/s/FragileXtesting

We would appreciate your response by *Friday, November 8, 2013*.

If you have comments, concerns, or questions about this research study, feel free to contact me.

Sincerely,

Lauren H. Brown, BA
Genetic Counseling Intern
University of Pittsburgh
(360) 606-4706
Lhb11@pitt.edu

### B.2.2 ASGC Recruitment E-mail

Dear ASGC members,

A Master of Genetic Counselling student in the US is conducting a survey exploring genetic counsellors' perceptions and utilization of AGG analysis to refine the risk of expansion in the FMR1 gene.

The research team is keen to include Australasian genetic counsellors in the survey and hopes that information gathered from this study will be of benefit to the genetic counselling profession and impact the utilization of genetic testing methodologies in the future. Your insight would be valuable, even if you do not regularly see patients with expansions in their FMR1 gene.

If you agree to participate, you will be asked to complete a brief, online survey. Completion of the anonymous survey should take no longer than 10 minutes. Simply click on the link below, or cut and paste the entire URL into your browser to access the survey:

https://www.surveymonkey.com/s/FragileX-ASGC

We would appreciate your response by Friday, December 20, 2013.

If you have comments, concerns, or questions about this research study, feel free to contact Lauren H. Brown, Genetic Counselling Student at the University of Pittsburgh, Lhb11@pitt.edu.

The study team includes:

Liane J. Abrams, MS, LGC (National Fragile X Foundation)
Alison D. Archibald, PhD., GDipGenetCouns. (Victorian Clinical Genetics Services)
Robin L. Bennett, MS, CGC, D. Sc. Hon. (University of Washington Medical Center)
Lauren H. Brown, BA (University of Pittsburgh)
Amy Cronister, MS (Integrated Genetics)
Brenda Finucane, MS, CGC (Geisinger Health System)
Allyn McConkie-Rosell, Ph.D. CGC (Duke University Medical Center)

Kind regards,

Lauren

Lauren H. Brown, BA
Genetic Counseling Intern
University of Pittsburgh
(360) 606-4706
Lhb11@pitt.edu

### B.2.3 AGNC Recruitment E-mail

Dear AGNC members,

I am a master of Genetic Counselling student in the US conducting a survey exploring genetic counsellors' perceptions and utilization of AGG analysis to refine the risk of expansion in the FMR1 gene.

The research team is keen to include genetic professionals from the UK in the survey and hopes that information gathered from this study will be of benefit to the genetic counselling profession and impact the utilization of genetic testing methodologies in the future. Your insight would be valuable, even if you do not regularly see patients with expansions in their FMR1 gene.

If you agree to participate, you will be asked to complete a brief, online survey. Completion of the anonymous survey should take no longer than 10 minutes. Simply click on the link below, or cut and paste the entire URL into your browser to access the survey:

https://www.surveymonkey.com/s/FragileX-UK

We would appreciate your response by Friday, November 8, 2013.

If you have comments, concerns, or questions about this research study, feel free to contact me.

The study team includes:

Liane J. Abrams, MS, LGC (National Fragile X Foundation)
Alison D. Archibald, PhD., GDipGenetCouns. (Victorian Clinical Genetics Services)
Robin L. Bennett, MS, CGC, D. Sc. Hon. (University of Washington Medical Center)
Lauren H. Brown, BA (University of Pittsburgh)
Amy Cronister, MS (Integrated Genetics)
Brenda Finucane, MS, CGC (Geisinger Health System)
Allyn McConkie-Rosell, Ph.D. CGC (Duke University Medical Center)

Kind regards,

Lauren

Lauren H. Brown, BA
Genetic Counseling Intern
University of Pittsburgh
(360) 606-4706
Lhb11@pitt.edu

## APPENDIX C: SURVEY FIRST DRAFT

1. Number of years in practice as a genetic counselor: __0-5 __6-10 __10-15 __>15

2. Do you see patients? __Yes __No

3. Specialty area(s): ___Prenatal ___Pediatric ___Cancer ___Adults genetics ___Other:_____

4. How many patients have you counseled who had a FMR1 mutation / intermediate allele over the past year? ___0 ___1-5 ___6-10 ___11-20 ___>20

5. On a scale of 1 to 5, with 1 being not at all knowledgeable and 5 being very knowledgeable, how would you rate your knowledge about the value of analyzing AGG interruptions in women with FMR1 intermediate or small premutation alleles? _____

If you rated yourself 2 or higher, please indicate how you learned and were educated about AGG analysis (Mark all that apply)
___ Attended a lecture on AGG analysis
___ Read peer review articles on the studies performed to date
___ Discussed it with a company or institutions that performs the testing
___ Other (Please Clarify): _____

6. When counseling patients with FMR1 intermediate or premutation alleles, how often do you discuss the role of AGG interruptions?
___Never ____Rarely ___Sometimes ___Almost always ___Always

7. When counseling patients with FMR1 intermediate or premutation alleles, how often do you offer follow-up AGG testing?
___Never ____Rarely ___Sometimes ___Almost always ___Always

8. If you have offered AGG testing, please indicate why testing was offered (Mark all that apply)
___ For further clarification of risk of expansion in a preconception or pregnant woman with an intermediate allele (45-54 CGG repeats)
___ For further clarification of risk of expansion in a preconception or pregnant woman with a premutation with 55-59 CGG repeats
___ For further clarification of risk of expansion in a preconception or pregnant woman with a premutation with 60-64 CGG repeats

___ For further clarification of risk of expansion in a preconception or pregnant woman with a premutation with 65-69 CGG repeats
___ For further clarification of risk of expansion in a preconception or pregnant woman with a premutation with 70 or higher CGG repeats
___ As part of a family study
___ As part of a research study
___To assist the patient in making a decision regarding prenatal diagnosis
___ To assist the patient in making decisions about family planning
___ Because it is standard of care
___ Because the patient requested AGG analysis
___ Other (Please Clarify ) _____

## APPENDIX D: SURVEY FINAL DRAFT

1. This survey is part of a research study to explore genetic counselors' perceptions and utilization of AGG analysis to refine the risk of expansion in the FMR1 gene. If you agree to participate, you will be asked to complete a brief, online survey. Completion of the survey should take no longer than 10 minutes.

   There is minimal risk involved in this study. All results generated through the electronic survey will be collected anonymously. Given the nature of the topic, it is possible that some questions may cause distress, as some individuals may feel uncomfortable thinking about the ethical implications of deciding whether or not to offer AGG testing to patients. Your participation in this study is completely voluntary and you may choose to exit the survey at any point.

   There are no costs to you for participating in this study, and you will receive no direct benefit from participating. Information gathered from this study will be of benefit to the genetic counseling profession and may impact the utilization of genetic testing methodologies in the future.

   If you have comments, concerns, or questions about this research study, you may contact Lauren Brown, BA by email at Lhb11@pitt.edu.

   If you have questions about your rights as a research participant, please contact the Human Subjects Protection Advocate at the University of Pittsburgh IRB Office, (866) 212-2668.

   I have read the above information and agree to participate in this research study.

   ___I accept the conditions ___I do not accept the conditions

2. Approximately how many years have you been in practice as a genetic counselor? ___

3. Specialty area(s) (Mark all that apply): ___Prenatal ___Pediatrics ___Cancer ___Adult genetics ___Research/Laboratory ___Other (Please Clarify) _____

4. Do you counsel patients? __Yes __No

5. Do you primarily work in the United States? __Yes __No
   a. If no, in which country do you primarily work? _____

6. Have you ever counseled a patient with an FMR1 intermediate or premutation allele? __Yes __No
   a. If yes, approximately how many patients have you counseled with an FMR1 intermediate or premutation allele within the past 3 years? ____

7. On a scale of 1 to 4, with 1 being not at all knowledgeable and 4 being very knowledgeable, how familiar are you with the notion of analyzing AGG interruptions in women with an FMR1 intermediate or premutation allele? _____

8. If you rated yourself 2 or higher, please indicate how you became educated about AGG analysis. (Mark all that apply)
   ___ Attended a lecture/presentation on AGG analysis
   ___ Viewed or participated in a webinar on AGG analysis
   ___ Read peer-reviewed articles on the studies performed to date
   ___ Read promotional materials from a company or institution that performs the testing
   ___ Discussed it with a company or institution that performs the testing

9. Has a patient ever asked you about additional testing (such as AGG analysis) to help refine their risk of expansion in the FMR1 gene? __Yes __No

10. When counseling patients with an FMR1 intermediate or premutation allele, how often do you discuss the role of AGG analysis to refine the risk of expansion in the FMR1 gene? ___Never ___Occasionally ___Often ___Always ___N/A

11. If you have offered AGG testing, please indicate why testing was offered. (Mark all that apply)
    ___ As part of a research study
    ___ Because it is standard of care
    ___ Because the patient requested AGG analysis
    ___ To assist the pregnant patient in making a decision regarding prenatal diagnosis
    ___ To assist the patient in making decisions about family planning
    ___ N/A – I have not offered this testing

12. Would you be more or less likely to utilize this testing methodology in the presence of family history consistent with possible Fragile X Syndrome, Fragile X-associated Tremor/Ataxia Syndrome, or Fragile X-associated Primary Ovarian Insufficiency? __More likely __Less likely

13. In the future, for which CGG repeat range would you be most likely to discuss the option of AGG testing for further clarification of risk of expansion? (Mark all that apply)
    ___ An intermediate allele (45-54 CGG repeats)
    ___ A premutation with 55-59 CGG repeats
    ___ A premutation with 60-64 CGG repeats
    ___ A premutation with 65-69 CGG repeats
    ___ A premutation with 70 or higher CGG repeats
    ___ None of the above, I do not plan to discuss this testing option with my patients

14. With which category do you most closely identify?
    ___ I have offered AGG analysis to patients in the past and will consider doing so again in the future
    ___ I have offered AGG analysis to patients in the past, however, I do not plan to offer this testing in the future
    ___ I have not offered AGG analysis to patients in the past, but would consider doing so in the future
    ___ I have not offered AGG analysis to patients in the past, and I do not plan to offer this testing in the future

15. Please answer the following true/false questions to help clarify what prevents you from offering/ordering AGG testing (if anything).

| | |
|---|---|
| True or False | I didn't know this testing was available and/or didn't know how to request testing |
| True or False | I need more education before I would feel knowledgeable enough to discuss this with my patients |
| True or False | I am concerned about insurance coverage and/or the additional cost this testing would add for my patient |
| True or False | I feel this information adds further complexity to FMR1 genetic counseling without actually changing patient decision-making |
| True or False | I feel this testing is not relevant for my patients with an FMR1 intermediate or premutation allele |

16. For patients with whom you discuss the role of AGG analysis to refine the risk of expansion in the FMR1 gene, approximately what percentage chooses to have the AGG analysis performed? ___ % ___ N/A – I do not discuss this

17. Should informing patients about the option of AGG analysis be the standard of care for genetics service providers? __Yes __No

18. Do you believe formal guidelines should be established for the purpose of identifying who should be offered AGG analysis? __Yes __No

19. If Yes, what organization(s) do you think should establish the guidelines? (Mark all that apply):
    ___ National Society of Genetic Counselors (NSGC)
    ___ American College of Medical Genetics (ACMG)
    ___ American Congress of Obstetrics and Gynecologists (ACOG)
    ___ American Academy of Pediatrics (AAP)
    ___ Other (Please Clarify) _____

20. Any additional comments about why you do/do not provide AGG analysis to help refine FMR1 gene expansion risk?

    Comments_____

Thank you very much for completing this survey. Your feedback is greatly appreciated.

## APPENDIX E: ADDITIONAL TABLES

**Table 16.** NSGC—Perceived Barriers Based on Familiarity II: Insurance

| I am concerned about insurance coverage and/or the additional cost this testing would add for my patient | | |
|---|---|---|
| | TRUE | FALSE |
| Knowledge Level 1 or 2 | 135 | 58 |
| Knowledge Level 3 or 4 | 97 | 60 |

Two-tailed Fisher's exact test: **p-value = 0.11**

**Table 17.** NSGC—Perceived Barriers Based on Familiarity II: Complexity

| I feel this information adds further complexity to FMR1 genetic counseling without actually changing patient decision making | | |
|---|---|---|
| | TRUE | FALSE |
| Knowledge Level 1 or 2 | 65 | 128 |
| Knowledge Level 3 or 4 | 51 | 106 |

Two-tailed Fisher's exact test: **p-value = 0.82**

**Table 18.** NSGC—Perceived Barriers Based on Familiarity II: Irrelevance

| I feel this testing is not relevant for my patients with an FMR1 intermediate or premutation allele | | |
|---|---|---|
| | TRUE | FALSE |
| Knowledge Level 1 or 2 | 20 | 173 |
| Knowledge Level 3 or 4 | 12 | 145 |

Two-tailed Fisher's exact test: **p-value = 0.46**

**Table 19.** NSGC—Perceived Barriers Based on Past Use: Insurance

| I am concerned about insurance coverage and/or the additional cost this testing would add for my patient | | |
|---|---|---|
| | TRUE | FALSE |
| Have offered AGG analysis to patients in the past | 41 | 27 |
| Have NOT offered AGG analysis to patients in the past | 191 | 91 |

Two-tailed Fisher's exact test: **p-value = 0.26**

**Table 20.** NSGC—Perceived Barriers Based on Past Use: Complexity

| I feel this information adds further complexity to FMR1 genetic counseling without actually changing patient decision making | | |
|---|---|---|
| | TRUE | FALSE |
| Have offered AGG analysis to patients in the past | 21 | 47 |
| Have NOT offered AGG analysis to patients in the past | 95 | 187 |

Two-tailed Fisher's exact test: **p-value = 0.67**

**Table 21.** NSGC—Perceived Barriers Based on Past Use: Irrelevance

| I feel this testing is not relevant for my patients with an FMR1 intermediate or premutation allele | | |
|---|---|---|
| | TRUE | FALSE |
| Have offered AGG analysis to patients in the past | 6 | 62 |
| Have NOT offered AGG analysis to patients in the past | 26 | 256 |

Two-tailed Fisher's exact test: **p-value = 1**

**Table 22.** NSGC—AGG Discussion Based on Familiarity: 45-54 repeats

| An intermediate allele (45-54 CGG repeats) | | |
|---|---|---|
| | Yes | No |
| Knowledge Level 1 or 2 | 70 | 76 |
| Knowledge Level 3 or 4 | 64 | 81 |

Two-tailed Fisher's exact test: **p-value = 0.56**

**Table 23.** NSGC—AGG Discussion Based on Familiarity: 55-59 repeats

| A premutation with 55-59 CGG repeats | | |
|---|---|---|
| | Yes | No |
| Knowledge Level 1 or 2 | 97 | 49 |
| Knowledge Level 3 or 4 | 109 | 36 |

Two-tailed Fisher's exact test: **p-value = 0.12**

**Table 24.** NSGC—AGG Discussion Based on Familiarity: 70+ repeats

| A premutation with 70 or higher CGG repeats | | |
|---|---|---|
| | Yes | No |
| Knowledge Level 1 or 2 | 89 | 57 |
| Knowledge Level 3 or 4 | 85 | 60 |

Two-tailed Fisher's exact test: **p-value = 0.72**

**Table 25.** Perceived Barriers for International vs. NSGC Respondents: Education

| I need more education before I would feel knowledgeable enough to discuss this with my patients | | |
|---|---|---|
| | TRUE | FALSE |
| International | 19 | 4 |
| NSGC | 237 | 113 |

One-tailed Fisher's exact test: **p-value = 0.1**

**Table 26.** Perceived Barriers for International vs. NSGC Respondents: Complexity

| I feel this information adds further complexity to FMR1 genetic counseling without actually changing patient decision making | | |
|---|---|---|
| | TRUE | FALSE |
| International | 7 | 16 |
| NSGC | 116 | 234 |

Two-tailed Fisher's exact test: **p-value = 0.82**

**Table 27.** Perceived Barriers for International vs. NSGC Respondents: Irrelevance

| I feel this testing is not relevant for my patients with an FMR1 intermediate or premutation allele | | |
|---|---|---|
| | TRUE | FALSE |
| International | 3 | 20 |
| NSGC | 32 | 318 |

Two-tailed Fisher's exact test: **p-value = 0.71**

**Table 28.** Standard of Care for International vs. NSGC Respondents

| Should informing patients about the option of AGG analysis be the standard of care for genetics service providers? | | |
|---|---|---|
| | YES | NO |
| International | 6 | 16 |
| NSGC | 155 | 193 |

One-tailed Fisher's exact test: **p-value = 0.08**

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