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Professional Issues

Genetic Counseling for Fragile X Syndrome: Updated Recommendations of the National Society of Genetic Counselors

Allyn McConkie-Rosell,[1,7] Brenda Finucane,[2] Amy Cronister,[3] Liane Abrams,[4] Robin L. Bennett,[5] and Barbara J. Pettersen[6]

These recommendations describe the minimum standard criteria for genetic counseling and testing of individuals and families with fragile X syndrome, as well as carriers and potential carriers of a fragile X mutation. The original guidelines (published in 2000) have been revised, replacing a stratified pre- and full mutation model of fragile X syndrome with one based on a continuum of gene effects across the full spectrum of FMR1 CGG trinucleotide repeat expansion. This document reviews the molecular genetics of fragile X syndrome, clinical phenotype (including the spectrum of premature ovarian failure and fragile X-associated tremor-ataxia syndrome), indications for genetic testing and interpretation of results, risks of transmission, family planning options, psychosocial issues, and references for professional and patient resources. These recommendations are the opinions of a multicenter working group of genetic counselors with expertise in fragile X syndrome genetic counseling, and they are based on clinical experience, review of pertinent English language articles, and reports of expert committees. These recommendations should not be construed as dictating an exclusive course of management, nor does use of such recommendations guarantee a particular outcome. The professional judgment of a health care provider, familiar with the facts and circumstances of a specific case, will always supersede these recommendations.

KEY WORDS: fragile X syndrome; genetic counseling; genetic testing; premature ovarian failure; FXTAS; premutation; FMR1; prenatal diagnosis; National Society of Genetic Counselors; practice guidelines.

PURPOSE

To present practice recommendations for genetic counselors and other health care professionals who provide genetic counseling and risk assessmentfor patients with suspected or confirmed fragile X syndrome and their families.

DISCLAIMER

The genetic counseling recommendations of the National Society of Genetic Counselors (NSGC) are developed by members of the NSGC to assist practitioners and patients in making decisions about appropriate management of genetic concerns. Each practice recommendation focuses on a clinical or practice issue and is based on a review and analysis of the professional literature. The information and recommendations reflect scientific and clinical knowledge current

[1]Duke University Medical Center, Durham, North Carolina.
[2]Elwyn Training and Research Institute, Elwyn, Pennsylvania.
[3]Genzyme Genetics, Phoenix, Arizona.
[4]Liane Abrams: National Fragile X Foundation, San Francisco, CA.
[5]Medical Genetics, Department of Medicine, University of Washington, Seattle, Washington.
[6]Genetic Counseling of Central Oregon, Bend, Oregon.
[7]Correspondence should be directed to Allyn McConkie-Rosell, PhD, CGC, Box 3525, Division of Medical Genetics, Duke University Medical Center, Durham, North Carolina 27710; e-mail: mccon006@mc.duke.edu.

as of the submission date and are subject to change as advances in diagnostic techniques, treatment, and psychosocial understanding emerge. In addition, variations in practice, taking into account the needs of the individual patient and the resources and limitations unique to the institution or type of practice, may warrant approaches, treatments, or procedures alternative to the recommendations outlined in this document. Therefore, these recommendations should not be construed as dictating an exclusive course of management, nor does use of such recommendations guarantee a particular outcome. Genetic counseling recommendations are never intended to displace a health care provider's best medical judgment based on the clinical circumstances of a particular patient.

METHOD

The authors consisted of experts in the field of genetic counseling for fragile X syndrome. Review and input was also sought from medical specialists with expertise in fragile X syndrome and patient advocacy groups. The authors searched the MEDLINE and PsycINFO databases for relevant English language medical and psychosocial literature between 1999 and 2004, including seminal articles from earlier dates. Key words included: fragile X syndrome, genetic counseling, psychosocial assessment gene testing, premature ovarian failure, prenatal diagnosis, carrier testing, and preimplantation diagnosis. Guidelines and policy statements published by the American College of Medical Genetics (Sherman, Pletcher, and Driscoll, 2005; Maddalena *et al.*, 2001), and genetic counseling guidelines developed by genetic counselors in the state of Washington (Marymee *et al.*, 1998) were also reviewed. This literature is based on clinical experience, descriptive studies and/or reports of expert committees. The literature was reviewed and evaluated for quality according to the categories outlined by the U.S Preventive Services Task Force (1995). The rating of supporting literature for this recommendation is class III: Opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees.

A draft document was made available to the 2072 members of NSGC for comment. The NSGC membership includes genetic counselors, physicians, nurses, attorneys, doctors of philosophy, and students. The revised document was reviewed by the NSGC attorney and the NSGC Ethics Subcommittee and no conflicts with the NSGC Code of Ethics or issues regarding legal liability were identified in the final document. The NSGC Board of Directors reviewed and approved the final document in March, 2005.

INTRODUCTION TO FRAGILE X SYNDROME

In 1969 Lubs reported the presence of an abnormal "marker X" chromosome in a family with males with mental retardation following an X-linked pattern (Lubs, 1969). It was not until 1977 that Sutherland was able to show that the expression of the marker X chromosome was inextricably linked to low folate concentrations in the cell culture medium (Sutherland, 1977). With this riddle solved, a relatively reliable cytogenetic test soon became available to distinguish the subgroup of males with the newly-named fragile X syndrome. Throughout the 1980s, as molecular advances put researchers within reach of the exact location of the fragile X gene, linkage analysis allowed relatively accurate carrier and prenatal testing for some families (Shapiro *et al.*, 1988). In 1991 the gene responsible for fragile X syndrome was identified (Oberle *et al.*, 1991; Verkerk *et al.*, 1991; Yu *et al.*, 1991), allowing highly reliable diagnostic, prenatal, and carrier testing. Despite these advances, several aspects of genetic counseling for fragile X syndrome remain challenging, including the interpretation of intermediate alleles and the widely variable clinical prognosis, particularly in females with fragile X mutations. Apart from the certainty that there is no male-to-male transmission of the fragile X mutation, genetic counselors should be wary of citing absolutes. As the understanding of the clinical phenotype in both males and females continues to evolve, the previously sharp clinical distinctions between pre- and full mutations have become more fluid. Recently, Hagerman and Hagerman (2004) proposed replacing the stratified pre- and full mutation model of fragile X syndrome with one based on a continuum of gene effects across the full spectrum of repeat expansion.

FMR1 Gene and FMR1 Protein (FMRP)

The FMR1 (Fragile X Mental Retardation-1) gene is characterized by a repetitive CGG trinucleotide sequence located in the 5′ promoter region, which, in most people in the general population, is repeated from 6 to 50 times. Two abnormal FMR1 states have been identified in association with fragile

X syndrome, both involving unstable expansions in the number of CGG repeats. *Premutation* alleles are unmethylated and have FMR1 gene sequences within the range of approximately 55–200 CGG repeats. Premutations are unstable in females, and may undergo further size expansions during oogenesis and postzygotic mitosis. CGG sequences with more than 200 repeats in number are considered *full mutations* and are associated with fragile X syndrome in both males and females. This may be reported as a smear indicating a range of repeat sizes all above 200. Mutations of this size are usually hypermethylated and do not produce FMR1 mRNA or protein. No instance of a child with a "new" FMR1 full mutation inherited from a parent with a normal size allele has been documented. Therefore, all mothers of children shown to have the full mutation are assumed to be obligate carriers of either a pre- or full FMR1 mutation.

The frequency of individuals with full mutations who are mosaic in lymphocytes for either the number of CGG repeats (size mosaics) or observed methylation pattern (methylation mosaics), is approximately 12% and 6%, respectively (Rousseau *et al.*, 1994a). *Methylation mosaics* have both methylated and unmethylated alleles on Southern Blot analysis. *Size mosaics* demonstrate a variety of allele sizes (full, premutation, or normal-sized alleles) on Southern Blot analysis.

The FMR1 gene in its normal state produces a protein that is thought to play a key role in both pre- and postnatal brain development. *Fragile X Mental Retardation Protein* (FMRP) is expressed in a variety of tissues, but it is most abundant in neurons (Devys *et al.*, 1993). Hypermethylated FMR1 full mutations inhibit FMRP production as a consequence of transcriptional repression (Pieretti *et al.*, 1991; Sutcliffe *et al.*, 1992), resulting in clinical symptoms. Based on research using the fragile X knockout mouse, it has been hypothesized that FMRP may play an important role in mGluR-mediated plasticity; a process by which dendrites in the brain mature (Bear *et al.*, 2004). FMRP may play an important role in maintaining the balance between how the brain strengthens (Long-Term Potentiation) or eliminates (Long-Term Depression) connections between neurons. The absence of FMRP results in unregulated activation of mGluR Long-Term Depression. Therefore, the brain is unable to establish and maintain strong synapses required for learning and memory. Research investigating the role of FMRP, mGluR, and other associated proteins is providing new hope for the treatment of fragile X syndrome and related disorders.

Prevalence

Although no consensus has been reached regarding specific prevalence figures for the fragile X full mutation, a range from 1 in 4000 to 1 in 6000 has been documented in population studies (Crawford *et al.*, 2002; Morton *et al.*, 1997; Turner *et al.*, 1996). The prevalence of affected males in cohorts of children with special needs ranges from .02% to 3% (Crawford *et al.*, 1999; De Vries *et al.*, 1997; Gonzalez-del Angel *et al.*, 2000; Hecimovic *et al.*, 2002; Kielinen *et al.*, 2004; Meadows *et al.*, 1996; Murray *et al.*, 1996; Syrrou *et al.*, 1998). The range may be explained by the populations studied and the diagnostic selection criteria used (e.g., special education classroom, autism, nonsyndromic mental retardation).

Rousseau *et al.* (1995) concluded that the prevalence of female carriers of an FMR1 premutation (>54 CGG repeats) is approximately 1 in 259, and the prevalence of male carriers of an FMR1 premutation is approximately 1 in 755. A study of over 14,000 women in Israel found 1 in 113 women had repeat lengths greater than 54 CGG repeats (Toledano-Alhadef *et al.*, 2001). The women screened had no known family history of mental retardation and were representative of the diverse Jewish population living in Israel. No systematic study has been done to accurately estimate the prevalence of the pre- or full mutation carriers in different ethnic or racial groups. Nevertheless, these figures provide some guidelines.

Diagnosing Fragile X Syndrome

The diagnosis of fragile X syndrome requires the detection of an alteration in the FMR1 gene at Xq27.3 (Verkerk *et al.*, 1991). Mutations resulting in an abnormal number of CGG trinucleotide repeats accompanied by abnormal methylation in the 5′ end of the gene are detected by PCR and Southern Blot analyses (see the section on FMR1 DNA Analysis) (Bell *et al.*, 1991; Fu *et al.*, 1991; Heitz *et al.*, 1991; Oberle *et al.*, 1991). Rare individuals with fragile X syndrome who have deletions of all or part of the FMR1 gene (Gedeon *et al.*, 1992; Tarleton and Saul, 1993; Wohrle *et al.*, 1992), or point mutations within it (De Boulle *et al.*, 1993), account for fewer than 1% of individuals with fragile X syndrome. The identification of deletions will vary depending on the probes used in Southern Blot analysis. To detect point mutations, however, direct sequencing of the FMR1 gene (which may only be available on a research basis) may be necessary.

FMR1 Instability and Factors Affecting CGG Repeat Expansion

The FMR1 gene contains a trinucleotide repeat, composed primarily of CGG, in the 5′ promoter region of the gene, outside the coding exons. Instability of the repeat is the predominant mechanism disrupting the expression of the FMR1 gene, accounting for >99% of symptomatic mutations (Kremer *et al.*, 1991; Oberle *et al.*, 1991; Verkerk *et al.*, 1991; Yu *et al.*, 1991). The number of CGG repeats at the FMR1 locus is variable in the general population, ranging from 6 to ~50 (Brown *et al.*, 1993; Fu *et al.*, 1991; Nolin *et al.*, 1996; Reiss *et al.*, 1994; Snow *et al.*, 1993). The most common number of repeats in an unexpanded FMR1 allele is 30 (Brown *et al.*, 1993; Snow *et al.*, 1993).

Individuals who carry premutation alleles are at risk of passing the unstable mutation to successive generations. The lower limit of the premutation range has been difficult to establish, but is thought to be somewhere around 55–60 CGG repeats. It is important to note, no expansion to a full mutation in one generation has been reported with alleles containing fewer than 59 CGG repeats (Nolin *et al.*, 1996, 2003).

A handful of studies (Table I) have examined factors affecting FMR1 gene expansion in females (e.g., Fu *et al.*, 1991; Nolin *et al.*, 1996, 2003; Sherman *et al.*, 1996; Snow *et al.*, 1993), and found that expansion of the CGG repeat is influenced by the gender of the carrier, the number of repeats, and the presence of AGG interruptions within the repeat. The process by which CGG repeat expansion occurs may be related to difficulties in DNA replication of the repeat. Premutations may undergo expansion during oogenesis in carrier females (Malter *et al.*, 1997), and during postzygotic mitosis in children who inherit the premutation from their mothers (Wohrle *et al.*, 1993). There are several reports of discordance in CGG repeat number and mental capacities between monozygotic twins (Cantu *et al.*, 1998; Helderman-van den Enden *et al.*, 1999; Tiberio, 1994). At this time, the exact mechanisms controlling the timing and extent of CGG repeat expansion remain unknown.

Intermediate Alleles

Intermediate or *gray zone* are the terms used to describe alleles that overlap the junction between the normal and premutation ranges (approximately 45–60) CGG repeats. Although alleles containing <55 repeats are generally considered stable, exceptions have been reported (Brown *et al.*, 1993; Crawford *et al.*, 2002; Nolin *et al.*, 1996; Reiss *et al.*, 1994; Sullivan *et al.*, 2002). Unstable repeats in the intermediate size range may be found in older generations (e.g. grandparent, great-grandparent) within fragile X families and expansion of an intermediate allele of 44 to a full mutation in two generations has been reported (Terracciano *et al.*, 2004). Because of the overlap of normal and premutation alleles in the intermediate range, it is difficult to interpret the significance of the intermediate size alleles when they are found in the general population. For this reason, results of a single test in this range should be interpreted in the context of the family and clinical history.

Multistep mutational models have been postulated to account for changes in trinucleotide repeat copy number from stable to unstable alleles (Ashley and Sherman, 1995; Kolehmainen, 1994; Morton and Macpherson, 1992). The risk for expansion of an intermediate allele to a premutation (greater than 55 CGG repeats) may be related to the absence of AGG interruptions, which influence the stability of the intermediate allele (Eichler *et al.*, 1994; Zhong *et al.*, 1996). Sequences of uninterrupted CGG repeats greater than 33–39 may increase the risk for instability of maternal alleles upon transmission to offspring (Eichler *et al.*, 1994; Kunst and Warren, 1994). This may explain why alleles with the same CGG repeat number in the intermediate range have different risks for instability. However, the presence of long, pure CGG tracts is not

Table I. Percent Expansion to Full Mutation with Transmission of Maternal Premutation Allele (Number of Expansions to Full Mutation/ Total Number of Pregnancies)

Maternal repeat size	Nolin *et al.*, 1996	Pesso *et al.*, 2000	Toledano-Alhadef *et al.*, 2001	Nolin *et al.*, 2003
55–59	13 (3/22)	0 (0/11)	0 (0/22)	4 (1/27)
60–69	21 (7/34)	12 (1/8)	10 (2/20)	5 (6/113)
70–79	58 (59/102)	50 (1/2)	17 (1/6)	31 (28/90)
80–89	73 (78/107)	50 (1/2)	...	58 (81/140)
90–99	94 (83/88)	100 (1/1)	...	80 (89/111)
100–200	99 (177/179)	75 (3/4)	...	98 (194/197)

Note. Data from Nolin *et al.* 2003.

necessarily sufficient to induce instability in the intermediate range (Nolin *et al.*, 1996). Furthermore, the determination of AGG repeats is not routine in most molecular diagnostic laboratories. Regardless of the mechanism, the risk for expansion to a full mutation lies in the succeeding generations, as the offspring of individuals with an intermediate allele, if inherited, will have either a similar size allele or a premutation. Mutations involving the trinucleotide repeat expansion from normal alleles of less than about 50 repeats to full mutations of >200 repeats are thought to be produced over several generations (Ashley and Sherman, 1995; Chakravarti, 1992; Kolehmainen, 1994).

Contractions

Reversion, or *contraction*, is the term used to describe the phenomenon whereby an individual who carries an expanded allele transmits a smaller-sized allele to his or her offspring. Contractions from premutations to normal-sized alleles have been documented in mother-to-daughter transmissions (Brown *et al.*, 1996a; Nolin *et al.*, 1996; Vits *et al.*, 1994), and approximately one-third of daughters of males who carry premutations have smaller premutations than their fathers (Fisch *et al.*, 1995). In addition, a study examining the sperm of males carrying full mutations found only alleles with premutation size repeats (Reyniers *et al.*, 1993).

CLINICAL PRESENTATION

Initially, individuals with fragile X mutations were divided into binary categories of affected (full mutations/methylated) and unaffected (premutations). The phenotypic range in individuals with full mutations has long been appreciated. However, as more is learned about the FMR1 mutation state, the clinical spectrum of symptoms associated with fragile X mutations should be seen as a continuum; individuals with premutations, as well as those with full mutations, present with different but relevant manifestations of this genetic condition.

Full Mutations

Males With Full Mutations

Males with full mutations may exhibit distinctive facial characteristics including large and/or protruding ears, a long face, prominent forehead, mandibular prognathism, strabismus, high arched palate with occasional cleft palate, and macrocephaly. The facial characteristics often develop over time, particularly the prominent forehead and chin (Fig. 1). Connective tissue findings include hyperflexible joints (particularly fingers, thumbs, and wrists), soft velvety skin, flat feet, and mitral valve prolapse. Genital abnormalities consist of macroorchism (testicles of more than 25 ml size) in postpubertal males.

The cognitive phenotype is characterized by a spectrum of features including developmental delay in the young child, mental retardation from mild to severe, borderline IQ, and learning disabilities. The behavioral phenotype includes attention deficit hyperactivity disorder (ADHD), speech and language delay, anxiety, hand flapping, hand biting with accompanying hand calluses, tactile defensiveness, sensory integration dysfunction, poor eye contact, perseverative speech, echolalia, and coprolalia. Autistic spectrum disorders are common.

Females With Full Mutations

In general, females with full mutations have milder features than males with full mutations but they also exhibit a similar range of cognitive, behavioral, facial, and connective tissue findings. Up to 50% of females with full mutations have some of the characteristic physical features associated with fragile X syndrome. Intellectual impairment is often milder in females than in affected males. Cognitive functioning can range from normal intelligence to learning disabilities to mental retardation. Studies indicate that approximately 53–71% of females with full mutations have IQs in the borderline or mentally retarded range (De Vries *et al.*, 1996; Rousseau *et al.*, 1991; Taylor *et al.*, 1994). Those females with full mutations who have normal IQs may have learning disabilities or emotional problems including social anxiety, selective mutism, shyness, poor eye contact, hyperactivity, and impulsive behaviors (Sobesky *et al.*, 1994; Keysor and Mazzocco, 2002). It is not uncommon for females to exhibit only subtle cognitive features, such as difficulty with math or excessive shyness without other major phenotypic effects (Cronister *et al.*, 1991).

Premutations

Both male and female carriers of premutations were previously considered to be clinically uninvolved. However, it is now known that these

Fig. 1. Two brothers, ages 4 and 6 years, with characteristic facial features of fragile X syndrome.

individuals may present with a spectrum of clinical findings including mild features of the fragile X syndrome, premature ovarian failure (POF), and fragile X-associated tremor/ataxia (FXTAS) (Hagerman and Hagerman, 2004).

Males With Premutations

Most males with premutations are unaffected by fragile X syndrome. However, there are rare reports of males with premutations who have mild manifestations, including physical, cognitive, and behavioral characteristics (Aziz, 2003; Tassone *et al.*, 2000). These manifestations may be due to somatic mosaicism in the target tissue.

Fragile X-associated tremor/ataxia syndrome (*FXTAS*), a recently identified neurological condition, primarily affects males over age 50 who carry the premutation (Hagerman *et al.*, 2001). FXTAS is a progressive neurodegenerative disorder characterized by intention tremor, cerebellar ataxia, Parkinsonism,

and peripheral neuropathy. Brain MRI studies of affected individuals are characterized by hyperintensities of the middle cerebellar peduncles (Hagerman *et al.*, 2001). The penetrance of FXTAS appears to increase with age (Jacquemont *et al.*, 2004). FXTAS may ultimately prove to be a common manifestation in males with premutations.

Females With Premutations

Females with premutations are usually unaffected intellectually and physically, although cases of females with premutations who are affected by cognitive and/or emotional disorders have been reported (Tassone *et al.*, 2000). Females with premutations may have an increased incidence of depression, social anxiety, and shyness (Franke *et al.*, 1998; Johnston *et al.*, 2001). More commonly, females with premutations are at increased risk for premature ovarian failure or ovarian dysfunction, accompanied by decrease in bone density (as observed in many postmenopausal women) (Hundscheid *et al.*, 2003; Schwartz *et al.*, 1994). (See the section on Reproductive Issues.) There have been case reports of females >50 years confirmed to have FXTAS (Hagerman *et al.*, 2004). However, the incidence, penetrance and severity of FXTAS in females with premutations remains unknown.

Males With Mosaicism (Methylation or Size)

(See the section on Genotype/Phenotype Correlation.)

Males and Females With Intermediate Alleles (45–54 CGG Repeats)

Individuals with intermediate size alleles are not generally considered to be at risk for clinical manifestations of either premutations or full mutations. Although there are rare reports of boys with intermediate alleles and some features of fragile X syndrome, it is uncertain whether the clinical manifestations in these boys are related to their genotype (Aziz, 2003).

Genotype/Phenotype Correlation

Although CGG repeat length does not correlate with severity, phenotype to genotype correlation is influenced by a number of variables, including the gender of the individual, methylation status, and tissue variation. X-inactivation should always be considered when discussing the phenotype in a female with a full mutation, as these individuals can show a full range of phenotypic findings, from normal intellectual function to mild learning disabilities to a phenotype similar to that found in full mutation males (De Vries *et al.*, 1996; Hagerman, 2002).

The degree of methylation has also been found to influence phenotypic expression (McConkie-Rosell *et al.*, 1993; Merenstein *et al.*, 1996). IQ scores of males with methylation mosaicism may be higher, on average, than scores of those with fully methylated mutations. Males with partially methylated premutation size alleles in the upper range may have mild clinical features. FMR1 protein may also be reduced in some individuals who have large premutation alleles, compared to protein levels of normal-sized alleles (Feng *et al.*, 1995; Hagerman *et al.*, 1994; Lachiewicz *et al.*, 1996; Rousseau *et al.*, 1994b; Smeets *et al.*, 1995). The biochemistry of FMR1 methylation is not well understood, but the existence of the above reports suggest that CGG repeat expansion and methylation are not absolutely coupled. As with any type of mosaicism, caution should be taken in interpreting results, as the repeat copy number in one tissue (e.g., blood, chorionic villi, amniocytes) may not reflect the methylation status and repeat copy number in others (de Graaff *et al.*, 1995). FMRP studies, which may only be available on a research basis, may be helpful in evaluating the impact of the mosaic pattern.

Currently, the penetrance of FXTAS as a function of CGG length is unknown; however this may be of great mechanistic importance and is currently under study (Jacquemont *et al.*, 2004). There may be a correlation between the number of CGG repeats and the risk for premature ovarian failure in females with premutations (Sullivan *et al.*, 2005). (See the section on Reproductive Issues.)

FMR1 DNA ANALYSIS

Methodology

The FMR1 gene can be analyzed using both Southern Blot and polymerase chain reaction (PCR) analysis. Combining both methodologies, testing is 99% sensitive in detecting affected and carrier individuals, missing only the rare individual who has fragile X syndrome because of a point mutation or

deletion located outside the CGG repeat region. Fetal testing performed on amniotic fluid cells or chorionic villi is also available when a parent is a known carrier (ACMG, 1994). (Prenatal diagnosis is discussed in the section on Prenatal Diagnosis.) A positive FMR1 test result is considered 100% specific. The exception to this is detection of a CGG repeat in the intermediate range (~45–54 CGG repeats), which may or may not be associated with fragile X syndrome in future generations. (See the section on Intermediate Alleles.)

Rousseau *et al*. (1991) were the first to publish methods for direct fragile X DNA diagnosis by Southern Blot analysis. To identify the FMR1 gene allele size(s) and methylation status, most laboratories use two restriction enzymes, one of which is methyl-sensitive, digesting only unmethylated DNA. Single and double digest of extracted DNA are considered reliable in detecting large expansions. Because of their ability to detect methylation, the use of two restriction enzymes is considered optimal for discriminating between premutations and full mutation alleles. A disadvantage of Southern Blot analysis is limited resolution, making it difficult to distinguish a normal-sized allele from a premutation allele and accurately determine of premutation allele size for risk assessment.

PCR analysis as described by Erster *et al*. (1992) and Brown *et al*. (1993) is the method of choice when measuring subtle differences in allele size, and can be used to distinguish alleles in the normal, intermediate, and premutation range. When used alone, PCR has the advantage of being less expensive than Southern Blot analysis, has a shorter turnaround time, and uses less DNA thereby allowing use of cheek brush or blood spot collection systems. However, most PCR methods cannot detect longer DNA sequences, as PCR amplification favors smaller allele sizes. Because of the limitations of both Southern Blot and PCR, most laboratories use both methods.

Results Interpretation

The American College of Medical Genetics published technical standards and guidelines for fragile X syndrome (Maddalena *et al.*, 2001) that outline recommended elements to include in the fragile X DNA report. In addition to stating the testing method(s), definitions of categories for normal and mutation (premutation, gray zone or intermediate, and full mutation) and the corresponding CGG repeat range should be included. Currently, there is variability in CGG repeat sizing between laboratories. Therefore, it is possible that a patient's results could be interpreted differently in separate laboratories when CGG repeats are within the borders of the normal, intermediate, and premutation range. Methods under development will enable standardization among laboratories and allow consistent detection of allele sizes that differ by as few as one triplet repeat.

Prenatal Diagnosis

Fetal testing performed on amniotic fluid cells or chorionic villi is available when a parent is a known mutation carrier (ACMG, 1994). A full discussion of each is described in the section on Prenatal Diagnosis.

TESTING AND SCREENING RECOMMENDATIONS

Testing Guidelines

The American College of Medical Genetics' Policy Statement on fragile X syndrome (Sherman *et al.*, in press) recommends fragile X testing for:

1) Individuals of either sex with mental retardation, developmental delay, or autism especially when associated with other physical and behavioral characteristics of fragile X syndrome, a family history of fragile X syndrome, or a relative with undiagnosed mental retardation.
2) Individuals with a family history of fragile X syndrome or a family history of undiagnosed mental retardation who are seeking reproductive counseling. When there is no established diagnosis of fragile X syndrome, testing the affected proband is preferable to screening an unaffected relative. However, this is not always feasible, especially in the prenatal setting.
3) Prenatal testing offered to individuals who are known FMR1 mutation carriers.
4) Individuals tested previously by cytogenetics who have results inconsistent with phenotype.
5) Women with reproductive or fertility problems associated with elevated FSH levels, especially if there is a family history of premature ovarian failure, fragile X syndrome, or undiagnosed mental retardation.
6) Individuals with late onset tremor or cerebellar ataxia of unknown origin, particularly

when there is a family history of movement disorders, fragile X syndrome, or undiagnosed mental retardation.

Testing Minors

Genetic testing in minors is a complex ethical and social concern. Current guidelines state that genetic testing of children is recommended only if a clear benefit to the minor can be demonstrated (ASHG/ACMG, 1995; National Society of Genetic Counselors, 1995). Special issues exist in counseling and testing minors which are covered in the section on Genetic Counseling Issues.

Population-Based Screening

Population-based screening has been proposed to aid in identifying carriers and individuals affected by fragile X syndrome. Although there is a growing awareness of fragile X syndrome and the importance of screening individuals with unexplained mental retardation, the majority of carriers remain unaware of their genetic status and reproductive risk (for review see Sherman, 2002). Discussions of population-based screening are complex because the implications of a positive test will vary depending on the population being screened. Similarly, the genetic counseling for individuals identified through population-based carrier or newborn screening would also be expected to differ based on how and when an individual was identified.

Palomaki (1994) and Finucane (1996) were among the first to evaluate the feasibility of general population screening for FMR1 status in women of reproductive age. They found that fragile X syndrome met population-based screening criteria, pointing out the diagnostic sensitivity of the DNA-based assay, but they warned that our inability to predict clinical status in female fetuses with full mutations was problematic and warranted further evaluation. Subsequent research studies among women of reproductive age have demonstrated the efficacy of fragile X screening programs in identifying female carriers and affected fetuses and concluded that carrier screening should be made more widely available to women in the general population (Pesso *et al.*, 2000; Ryynanen *et al.*, 1999; Toledano-Alhadef *et al.*, 2001). Another publication (Musci and Caughey, 2003) used decision tree analysis and predicted that population-based fragile X screening of pregnant women would identify 98% of fetuses affected with fragile X syndrome annually and may be cost-effective. A study by Skinner and colleagues (2003), examining the attitudes and perspectives of families with fragile X syndrome regarding screening, found that parents were generally supportive and saw benefits in making voluntary carrier screening for fragile X syndrome available more broadly.

The genetic counseling that accompanies population screening is critically important; there is some evidence that without sufficient pre-test education, women from the general population would be wholly unprepared for positive carrier results (Anido *et al.*, 2005), and there are limited data on the psychological impact of positive fragile X carrier results among women at population risk to be FMR1 mutation carriers.

A common concern frequently expressed regarding FMR1 population-based screening is the issue of identifying women with intermediate alleles. One study reports the intermediate allele frequency among women to be as high as 1 in 52 (Murray *et al.*, 1996). Critics of widespread carrier screening for fragile X syndrome cite this high intermediate allele frequency, and the presumed association with increased anxiety and increased cost of testing, as a reason not to implement such programs. To respond best to this issue, protocols regarding identification and management of intermediate allele carriers as well as patient education programs outlining the implications of carrier testing should be developed prior to implementing any population-based carrier screening program.

Newborn screening for fragile X syndrome is under consideration. Supporters argue that early detection leads to early intervention and improved outcomes (Bailey, 2004; Skinner *et al.*, 2003). While there are no data to confirm that early intervention affects the long-term outcome of fragile X syndrome, there is much anecdotal evidence with fragile X syndrome and other disabilities to support the importance of early intervention. Currently, however, newborn screening for fragile X syndrome does not meet all of the established screening criteria (Ciarleglio *et al.*, 2003; website US General Accounting Office, 2003). And, although likely on the horizon, an inexpensive screening test will need to be developed. Research is necessary to investigate the medical, family, economic, and ethical perspectives, and to delineate the advantages and disadvantages of newborn screening for fragile X syndrome.

The recently revised ACMG policy statement on fragile X syndrome (Sherman *et al.*, in press) supports testing women with a family history of

undiagnosed mental retardation. However, population carrier screening was not recommended by the ACMG, except as part of a well-defined research protocol. The ACMG policy statement cautioned against general population-based screening until the complex genetic counseling issues including the broad phenotypic expression of the FMR1 mutation (including FXTAS) have been adequately addressed on a clinical level.

REPRODUCTIVE ISSUES

Reproductive concerns for individuals with the fragile X mutation vary based on the number of CGG repeats yielding differences in fertility, prenatal diagnostic options, and genetic risk. Risk of expansion of the unstable mutation is discussed in the section on FMR1 Instability and Factors Affecting CGG Repeat Expansion. This section focuses on fertility, preconception options, prenatal diagnostic options, and the implications for genetic counselors.

Fertility Issues

Females

Premature ovarian failure (POF) is defined as menopause occurring prior to the age of 40. The risk of POF has thus far been found only in carriers of the FMR1 premutation and not in carriers of the full mutation. Of women identified through families with fragile X syndrome, approximately 13–24% of women who are premutation carriers have POF (Allingham-Hawkins *et al.*, 1999; Sherman, 2000; Sullivan *et al.*, 2005). Conversely, premutation alleles have been identified in 2% of women with idiopathic sporadic POF and in 14% of women with a family history of POF and no known history of fragile X syndrome (Sherman, 2000). It is unknown whether the risk of POF is higher in women with premutations who have a family history of POF than in those who do not.

Although a parent-of-origin effect regarding the relative risk of POF has been reported (Hundscheid *et al.*, 2000) subsequent studies have failed to confirm this observation (Murray, Ennis, and Morton, 2000b; Sullivan *et al.*, 2005; Vianna-Morgante and Costa, 2000). There is no apparent correlation between the age at menopause and the X-inactivation pattern among carriers of the premutation (Murray *et al.*, 2000a). There may be a correlation between the number of CGG repeats and the risk for premature ovarian failure in females with premutations (Sullivan *et al.*, 2005). However, this relationship appears to be nonlinear. Sullivan *et al.* (2005) suggest the risk for POF may increase with increasing size repeat in premutation carriers in the range of 59–99, but that the risk may decrease for women with larger premutation size alleles (>100).

The etiology of POF in carriers of the premutation is not known. It has been hypothesized that POF is secondary to increased levels of the FMR1 transcript (Conway *et al.*, 1995) observed in carriers of the premutation (Tassone *et al.*, 2000). Carriers of the full mutation are thought to be unaffected because they produce mRNA only from their normal allele.

The risk of POF has significant reproductive implications. The onset is insidious and difficult to predict. The possibility of early menopause leading to reduced fertility should be included in the genetic counseling of women identified with a premutation. Additionally, the ovarian dysfunction also reduces the chances of a successful pregnancy using preimplantation diagnosis secondary to a low yield of available eggs (Platteau *et al.*, 2002). Additionally, there is evidence that premutation carriers may have hormonal changes suggestive of early ovarian aging despite regular menstrual cycles (Welt *et al.*, 2004).

Males

Males with either the full mutation or the premutation do not appear to have reduced fertility. Interestingly, the sperm of males with full mutations have only premutation size repeats (Reyniers *et al.*, 1993). Although both pre- and full mutation males are expected to transmit premutation size alleles to their daughters, there has been one case report of a male with mosaicism who transmitted a full mutation to his daughter (Zeesman *et al.*, 2004).

Preconception Options

Individuals at risk for passing on fragile X mutations to their offspring have a variety of pre- and postconception options available. Some couples may consider adoption in order to bypass the genetic risk. Pregnancy can be achieved using donor eggs or sperm for female and male mutation carriers, respectively. Given the relatively high prevalence of fragile X syndrome in the general population, potential gamete donors should be screened for fragile X mutations.

Males at risk for passing on fragile X premutations to their daughters may wish to consider sperm-sorting techniques for sex selection of males. The efficiency and reproducibility of these techniques are controversial. Sex selection of spermatozoa by chromatin differences has achieved significant enrichment of X- or Y-chromosome bearing sperm, but clinical experience in humans remains limited (Sills *et al.*, 1998).

Preimplantation genetic diagnosis (PGD) for fragile X syndrome is possible but should be approached with caution. PGD for fragile X syndrome can be performed on either the polar body or on biopsied embryos. Difficulties have been encountered in oocyte retrieval, number of viable embryos, the ability to distinguish alleles, and amplification of the CGG repeat (Platteau *et al.*, 2002). In their single center study of PGD for fragile X syndrome, Platteau *et al.* (2002) found that the volume of eggs retrieved was reduced, and only 55% of fertilized oocytes reached the stage of embryo biopsy, thus leading to a significantly reduced opportunity for pregnancy. Given the potential for premature ovarian failure, premutation carriers should be evaluated for subfertility prior to consideration of PGD.

Linked polymorphic markers and direct detection of the expanded CGG repeat have both been used for PGD (Apessos *et al.*, 2001; Sermon *et al.*, 1999). Because the expanded CGG repeat is technically difficult to amplify using PCR techniques, preimplantation diagnosis is based on the presence of the normal maternal allele. Prior to attempting PGD, paternal and maternal allele sizes should be determined by direct detection of CGG repeats, as well as linked polymorphic markers when allele sizes differ by one or zero, to ensure that the couple is informative.

Prenatal Diagnosis

Amniocentesis and Chorionic Villus Sampling

Prenatal diagnostic options for fragile X syndrome include amniocentesis and chorionic villus sampling (CVS). Amniocentesis is both accurate and reliable using the combined standard DNA diagnostic methods of Southern Blot and PCR (Brown, 2002; Brown *et al.*, 1996b). The methylation status of the FMR1 region and the number of CGG repeats in the fetus can be accurately determined in amniocytes. Prenatal detection of the CGG repeat number for fragile X syndrome using CVS is accurate and reliable; however, there are special considerations that should be taken into account regarding the degree of methylation of the placental tissue. The methylation pattern observed in placental (CVS) tissue at 10–12 weeks gestation is incomplete and does not always reflect that observed in the liveborn (Iida *et al.*, 1994; Willemsen *et al.*, 2002). Because the clinical phenotype is influenced by both the number of CGG repeats and the degree of methylation, it can be difficult to distinguish large unmethylated premutations and small methylated full mutations. The possibility of follow-up amniocentesis to clarify the status of the fetus, if the CVS result is indeterminate, should be discussed as part of the pretest counseling. For both amniocentesis and CVS, it may be helpful to determine both maternal and paternal allele number either prior to or concurrent with the prenatal testing. PCR analysis of fetal and parental DNA can be useful in assessing the fetal genotype prior to completion of the Southern analysis.

GENETIC COUNSELING ISSUES

Comprehensive genetic counseling for individuals and families in whom the diagnosis of fragile X syndrome is suspected or has been made may require several sessions and may involve a long-term commitment on the part of the genetic counselor to follow these families. If this commitment cannot be made, referring the family to a genetic counselor or center experienced with fragile X syndrome should be considered.

The general assessment (medical, family, and psychosocial histories; risk assessment), genetic counseling, management, and follow-up processes pertinent to fragile X syndrome are similar to those outlined in previous NSGC practice guidelines (Bennett *et al.*, 2002; McIntosh *et al.*, 2000; Trepanier *et al.*, 2004). Genetic counseling for fragile X syndrome should follow the recommendations in these guidelines with special attention given to genetic counseling methods and issues associated with X-linked disorders (Bennett *et al.*, 2002; McIntosh *et al.*, 2000). Issues specific to genetic counseling for fragile X syndrome are outlined in the following section.

Diagnostic Evaluation

When obtaining family, medical, and psychosocial histories from patients and families, follow standard genetic counseling practice recommendations (refer to www.ngc.gov). Targeted medical family

Table II. Suggested Targeted Family History Questions for Fragile X Syndrome

Note for each relative, any history, and the age of onset of:
• Cognitive effects: Mental retardation, developmental delay, learning disabilities, specific problems with math.
• Speech delay or unusual speech pattern.
• Autistic spectrum disorders or autistic-like behaviors (gaze avoidance, repetitive behaviors, hand-flapping, hand biting, touch avoidance, etc.).
• Attention deficit disorder (ADD) or attention deficit hyperactivity disorder (ADHD).
• Dysmorphic features—macrocephaly, large ears, long face, broad forehead, prominent jaw, strabismus.
• Features of loose connective tissue: hyperextensible joints, flat feet, hypotonia, mitral valve prolapse, large testicles, hernias, recurrent ear infections.
• Neurologic symptoms: seizures, late-onset progressive tremor, ataxia, difficulty walking, balance problems, short-term memory loss, loss of sensation in limbs.
• Mental illness/personality disorders: depression, schizophrenia, bipolar disorder, obsessive-compulsive disorder, schizoaffective disorder, schizoid personality, etc.
• Behavioral problems: impulsiveness, anger outbursts, violent behavior, solitary behavior, counseling or medication for behavioral difficulties.
• Shyness, social anxiety, excessive worrying, counseling or medication for emotional difficulties.
• Premature menopause, fertility problems.

history questions specific to fragile X syndrome are included in Table II and are appropriate for use in cases of suspected fragile X syndrome, families with a confirmed diagnosis, and at-risk or known carriers.

Confirmed Diagnosis of Fragile X Syndrome

Genetic counseling sessions for families with newly diagnosed fragile X syndrome offer opportunities for education, counseling, and guidance regarding the issues and concerns specific to fragile X syndrome. Particular challenges inherent in genetic counseling for fragile X syndrome include:

- the extremely variable expression of the disorder, especially in females with the full mutation;
- the concepts of intermediate, premutation, and full mutation alleles and the mechanism of expansion including the multigenerational mutation process and complexity inherent in understanding the concept of carrier males in an X-linked disorder;
- the variable recurrence risks based on size of the premutation in the female carrier;
- the recent findings of FXTAS and POF in carriers of the premutation.

Genetic counselors should to be cognizant of the fact that, in general, families are initially overwhelmed, both emotionally and intellectually, by the complexity of the disorder and its implications for other family members. Suggested components for genetic counseling sessions for families with a suspected or confirmed diagnosis of fragile X syndrome appear below.

Education/Health Promotion

1. Discuss the clinical presentation and natural history of fragile X syndrome in males and females.
2. Discuss the inheritance pattern and genetics of fragile X syndrome and the approach to testing the proband and other family members and interpretation of results.
   a. FMR1 testing, including a discussion of the CGG repeat, methylation, sensitivity, and specificity.
   b. X-linked inheritance pattern, including examples of females and males with the premutation and full mutation, and the risk of expansion/reversion in such cases.
   c. Reproductive options and testing available to fragile X carriers (e.g., adoption, donor egg or sperm, prenatal diagnosis, preimplantation genetic diagnosis); include ethical concerns raised by such options, if appropriate.
   d. Costs of genetic testing and test limitations (e.g., limitations of CVS).
3. Be prepared to answer general questions relating to suggested treatment, therapy, and the function of the FMR1 protein.
4. Discuss follow-up recommendations (e.g., identification and testing of at-risk family members, scheduling follow-up visits).

Table III. Printed and Online Resources on Fragile X Syndrome for Patients and Professionals

Family-oriented support groups
National Fragile X Foundation., http://www.fragilex.org P.O. Box 190488 San Francisco, CA 94119-0988 Phone: 800-688-8765
FRAXA Research Foundation, http://www.fraxa.org 45 Pleasant St. Newburyport, MA 01950 Phone: 978-462-1866
Family-oriented literature
Braden, M. L. (2000). *Fragile Handle with care: More about fragile X syndrome—adolescents and adults*. Dillon, CO: Spectra. Finucane, B., McConkie-Rosell, A., & Cronister, A. (2002). *Fragile X syndrome: A handbook for families and professionals*. San Francisco, CA: Elwyn Inc., and the National Fragile X Foundation. Harris-Schmidt, G., and Fast, D. (2004). *The source for fragile X syndrome*. East Moline, IL: LinguiSystems. Weber, J.D. (2000). *Children with fragile X syndrome: A parents' guide*. Bethesda, MD: Woodbine House.
Other online resources
The Arc's Q&A on fragile X syndrome. Available at http://www.thearc.org/pdf/gbr05.pdf National Institute of Child Health and Human Development. *Families and Fragile X*. Available at http://www.nichd.nih.gov/publications/pubs/fragileX/index.htm *Your genes your health: Fragile X syndrome*. Available at http://www.ygyh.org/fragx/whatisit.htm
Resources for health professionals
GeneClinics. Availabe at www.geneclinics.org *GeneReviews*. Availabe at www.genereviews.org (enter "Fragile X") Hagerman, R. J. (2001). Fragile X syndrome. In S. B. Cassidy & J. E. Allanson (Eds.), *Management of genetic syndromes* (pp. 165–183). New York: Wiley-Liss. Hagerman, R. J., & Hagerman, P. J. (2002). Fragile X syndrome: Diagnosis, treatment, and research (3rd Ed.). Baltimore, MD: The Johns Hopkins University Press.

5. Make appropriate referrals for medical, educational, and mental health interventions and discussions that are beyond the scope of genetic counseling practice.
6. Provide contact information for support groups and patient-appropriate resources, as requested (Table III).

Risk Assessment

Analyze the pedigree and FMR1 DNA results and provide genetic risk assessment for carrier status and chance of having affected or carrier offspring. Inheritance principles for fragile X syndrome include:

1. All daughters of a male with a premutation are obligate premutation carriers, whereas none of his sons will inherit the mutation.
2. Females with premutations and full mutations are at risk to have affected sons and daughters.
3. The risk for affected offspring in females carrying premutations varies with the length of the repeat number (Table I).
4. Women with full mutations have a 50% risk with each pregnancy to pass the full mutation to the fetus. Although rare, there are reports of women with full mutations having offspring with reversions (decreases in the number of the repeat) or premutation-sized alleles (Loesch *et al.*, 1995; Nolin *et al.*, 1996).
5. Males with a full or mosaic mutation will not pass it on to their sons and most likely will pass on a premutation to their daughters. Though it was previously thought that all males with full mutations have only premutations in their sperm, there are rare reports of daughters with full mutations born to males with full/mosaic mutations.

Informing Family Members

The diagnosis of fragile X syndrome can have far-reaching genetic and emotional implications for extended family members. Newly-identified mutation carriers as well as families who have been previously diagnosed usually benefit from discussion of strategies for disclosing information about fragile X syndrome

to other relatives, some of whom may react with anger, guilt, blame, disbelief, or indifference. Difficult ethical situations arise when key family members refuse to relay information about fragile X syndrome to at-risk relatives.

Because of the difficulty frequently encountered when informing relatives of genetic risk, genetic counselors should work with clients to develop a strategy to inform relatives as part of initial as well as follow-up genetic counseling sessions. Genetic counselors can assist families by identifying at-risk relatives in the pedigree and reviewing strategies for broaching the subject of diagnostic or carrier testing. Utilization of a family network approach, which allows relatives to initially be informed by a family member known to them with follow-up by a genetic counselor, may be helpful in facilitating informing relatives about their genetic risks (McConkie-Rosell *et al.*, 1995). Families should be reassured that it is not their responsibility to provide in-depth genetic counseling or ensure that other family members pursue testing. Families with fragile X syndrome often find it helpful to have an objective document, such as a succinct summary letter with the genetic counselor's contact information, to give to other relatives at the time of disclosure. As always, genetic counselors dealing with different branches within a family should be careful to maintain confidentiality and avoid revealing clinical and diagnostic information without the consent of those involved.

SPECIAL ISSUES REGARDING FMR1 CARRIER TESTING

Family History of Mental Retardation of Unknown Etiology

Individuals with family history of mental retardation of unknown etiology (e.g., an affected proband is not available for testing) should be offered fragile X carrier testing after counseling and education about fragile X syndrome. Implications of results of carrier testing, including available methods of prenatal diagnosis and possible results and their meaning should also be discussed.

Women With a Positive Family History

All women with a family history of fragile X syndrome who have been determined by pedigree analysis to be at risk to be carriers should be offered genetic counseling, including an informed consent process, prior to carrier testing (Bennett *et al.*, 2002; McIntosh *et al.*, 2000). The carrier testing process in fragile X syndrome has been studied from the perspectives of at-risk women (McConkie-Rosell *et al.*, 2000, 2001, 2002), obligate carriers (McConkie-Rosell *et al.*, 1997), and parents of children with fragile X syndrome (McConkie-Rosell *et al.*, 1999). Findings from this research can be used to develop genetic counseling interventions.

The effect on self-concept related to the carrier testing process has been studied in adult women at 50% risk for inheriting the fragile X mutation. While overall self-concept was found to be stable, feelings about self related to the implications of "being a carrier" were negatively affected. Five areas of concern were identified:

- implications of a positive carrier test for their children
- a barrier to having biological children or grandchildren
- possible expression of clinical features of fragile X syndrome in themselves
- a heightened awareness of their genetic identity
- regret over not having learned this information sooner.

The decreased positive feelings about self and the coping behaviors to manage them were present in *all* the women when they were "at-risk" and persisted in those women subsequently found to be mutation carriers (McConkie-Rosell *et al.*, 2001). These findings suggest that pretest genetic counseling interventions should include assessment of the coping behaviors used to manage feelings related to "being at risk" and facilitation of positive coping skills to manage carrier test results. Coping resources include physical resources (e.g., family finances, job skills, education, etc.), social and family support networks, and psychological resources such as beliefs, cognitive skills, problem solving abilities, and self-concept. The adaptive coping behaviors identified in response to genetic testing in adults include: pursuing hope, constructing meaning, acquiring new knowledge and coping methods, minimization, and perceived control (Kessler *et al.*, 1984; Marteau *et al.*, 1997; McConkie-Rosell *et al.*, 2001; Shiloh *et al.*, 1997).

Daughters of Males With Premutations

Test results for daughters of a male with a premutation should not be inferred from their father's

results. There is a possibility of misattributed paternity or of gene reversion. Additionally, testing obligate carriers may help them to better understand their *own* genetic status.

Males With a Positive Family History

FMR1 testing in males has become more complicated with the discovery of FXTAS. The newly described clinical complication in premutation carriers means that carrier testing may uncover a risk of unknown magnitude for FXTAS later in life. Therefore, presymptomatic testing concerns may apply. Additional epidemiological data are needed in order to determine the age-related risk for FXTAS. Carrier testing in males occurs under several different circumstances: testing of maternal grandfathers; concerns regarding reproductive risk; and parental request for testing secondary to educational or behavioral concerns in a son. Carrier males may also be diagnosed through prenatal testing. Each of these different circumstances has unique implications that affect the risks and benefits of testing which should be considered in genetic counseling.

Once the diagnosis of fragile X syndrome is made in the family, testing of the maternal grandparents is often recommended in order to determine which side of the family is at risk. The grandfathers of an affected child are often close to the age of onset of FXTAS or they may already be symptomatic. For these men, DNA carrier testing may become diagnostic testing, and referral for neurological evaluation may be appropriate.

Male relatives of an affected child may request FMR1 testing for reproductive purposes because males with premutations are at risk to have daughters who are premutation carriers. Therefore, genetic counseling should include a discussion regarding the issues of informing a daughter about her genetic risk (see the section on "Minors," below) and the potential clinical implications.

Testing may also be requested by parents because of an educational or behavioral concern in their minor son. Careful consideration and discussion of the risks and benefits of carrier testing to the male in question should be the focus of the genetic counseling session (as noted below). For carrier males identified through prenatal screening genetic counseling should focus on helping the family to determine when and how to inform about the genetic risk.

Minors

Fragile X carrier testing for children less than 18 years of age must be approached carefully, with medical and emotional benefits to the child weighed against potential harms (Table IV).

Research with parents of children with fragile X syndrome (McConkie-Rosell *et al.*, 1999) and with obligate carriers of fragile X syndrome (McConkie-Rosell *et al.*, 1997) suggests that families are concerned about when and how to tell their children they could be carriers; how to weigh the potential risks and benefits of carrier testing for their own family; and how to help family members positively adapt to this information. Addressing how families should provide this information to their children is complicated and usually requires ongoing discussion. The genetic counseling should focus on the adjustment to genetic

Table IV. Risks and Benefits of Testing a Minor for Carrier Status

Potential adverse consequences of testing a minor
• Damage to the minor's self-esteem.
• Distortion of the family's perception of the child.
• Siblings may be treated differently depending on genetic status.
• Loss of future adult autonomy and confidentiality for the tested child.
• Adverse effects on the child's capacity to form future relationships.
• Fear/guilt if person wants biological children.
• Discrimination (insurance, employment, education, choice of mate).
Potential benefits of testing a minor
• Resolution of the parent's (and possibly the child's) concerns about carrier status.
• Allows child and family time to adjust to test outcome and to develop coping behaviors.
• Genetic counseling can be tailored to the developmental stage of the child and anticipatory guidance provided for future concerns.
• Child and parents can be informed of genetic risk prior to the occurrence of an unintended pregnancy.
• Allows for long-term integration of information regarding genetic status for family planning issues.
• Awareness of risk of premature ovarian failure allows decision-making regarding timing of future pregnancies.

risk status throughout the life cycle. How this information is managed when a child is young is critical and will influence how the child adjusts to his/her genetic risk and copes with that information as an adult.

Genetic counseling interventions should be tailored to the developmental stage of the child (McConkie-Rosell *et al.*, 2002), and consideration made to use a family approach in order to facilitate problem solving and open discussion (McConkie-Rosell and Spiridigliozzi, 2004). Genetic counselors need to be prepared to work with the parents to develop a plan for how to approach talking with children about genetic risk and carrier testing, and also be able to facilitate the discussions between parents and their children. Topic areas to discuss in the development of a plan include:

- How, when, and why parents want to talk with their children about the genetic risk.
- Parents should be encouraged to think about what "message" they are trying to convey to their children about fragile X syndrome, how it is inherited, and what "being a carrier" means.
- Parents should be encouraged to take their time in considering what they want to say and be able to discuss genetic risk without overwhelming the child with facts or emotion.
- Parents should also be aware that their children's needs and understanding may change over time and discussions may need to be repeated to address misunderstandings or changes in their child(rens)'s needs. Genetic counselors are encouraged to plan with the parents potential times for follow-up counseling sessions to address new issues.

Once a plan has been developed, genetic counselors can facilitate discussions between parents and their children. Using age and developmentally appropriate words, counseling with children should include:

- What have they been told and what do they understand about fragile X syndrome, in general?
- What do they understand about how fragile X syndrome is inherited?
- What do they understand about their risk for being a carrier? (If their status is known, e.g., a daughter of a carrier male, what does "being a carrier" mean to them?)
- Are they interested in being tested?
- Do they understand what the test results might mean?

In summary, there are both risks and benefits to pursuing carrier testing in minors. Discussion of all potential consequences should occur prior to decisions about testing. It is important to adapt the above genetic counseling approaches for minors identified as premutation carriers through prenatal diagnosis and for minor daughters who are assumed to be obligate carriers because of paternal transmission of an FMR1 premutation.

Counseling Issues for Carriers

Premature Ovarian Failure

Although the incidence of POF in females who are carriers of the premutation has been found to be about 20% for women under the age of 40 years, the specific incidence in young women between the ages of 20 and 35, who may be actively making reproductive plans or who are not yet ready to consider their reproduction, has not yet been established. Carriers of the premutation who are in this latter category may be faced with altering their life plans related to child bearing. Female carriers of the premutation should be informed about the potential for reduced fertility. However, similar to counseling young girls with Turner syndrome, care should be taken to present a balanced picture of the potential for reduced fertility in the context of life decisions and timing for reproduction (Sybert, 2001). Although surveillance for the clinical onset of POF is difficult, it may also be helpful to recommend close medical follow-up for early signs of POF.

Family Planning Issues and Options

In addition to providing factual information regarding reproductive options, genetic counseling for families managing the genetic risk for fragile X syndrome may also require helping the client reframe the parental role (McConkie-Rosell and DeVellis, 2000). Fundamental concepts of the parental role, including how it is defined and fulfilled may need to be reevaluated due to the potential barrier to reproduction inherent in the genetic risk. In this regard, the genetic counseling should include discussion of different ways to fulfill the parental role (e.g., adoption, foster care, remaining childless or no further children, parenting a child with fragile X syndrome, and prenatal testing options), exploration of the couple's/family's personal

definition of what being a parent means and how important this role is to them.

Females With Full Mutations

Carrier testing may reveal that a female has either a premutation or a full mutation allele. It is important to be aware that some cognitively normal females with a family history of fragile X syndrome may have the full mutation. The possibility of either the pre- or full mutation and the implications of each should be addressed prior to carrier testing.

Psychiatric and intellectual disabilities related to fragile X mutations can also adversely affect the genetic counseling process. Mental retardation, concreteness, inconsistent attitudes, and tangential thinking in some women may limit the success of traditional genetic counseling approaches. Women with cognitive and psychological impairments may benefit more from exploration of feelings and attitudes rather than an education-based model of genetic counseling (Finucane, 1998a, 1998b).

Premutations and Predisposition to Psychological Issues

Psychological issues such as denial, anxiety, anger, grief, survivor and parental guilt, shame, blame, depression, inability to cope, damage to self-esteem, changed relationship with family of origin, and change in sense of identity are potential reactions to any X-linked disorder (Baker *et al.*, 1998; Bennett *et al.*, 2002; Resta, 2000; Weil, 2000; Williams *et al.*, 2000).

Complicating the reactions to the diagnosis or carrier status itself can be one or more of the psychological components inherent to a proportion of premutation carriers (see the section on Clinical Presentation). Risks for these conditions should be discussed and, if symptoms or signs present, appropriate referrals to mental health professionals should be made.

Prenatal Diagnosis and Genetic Counseling

Prenatal diagnosis should be offered to women identified as carriers of a pre- or full mutation. Males identified as premutation carriers, and therefore at risk to have premutation daughters, should also be presented with the benefits and limitations of an invasive procedure and the implications of prenatal results and be allowed to make the choice that is right for them. As with other genetic conditions, it is the role of the genetic counselor in the prenatal setting to fully explain the implications of different test results, including the range of possible outcomes for a female fetus with a full mutation. The variable phenotype among males and females with premutations should also be emphasized. This information, as well as information regarding the benefits and limitations of an invasive procedure, can facilitate patient decision-making and help prepare patients who ultimately are faced with a positive result.

Carrier Testing for Reasons Other Than Fragile X Syndrome

Increasingly individuals are being referred for fragile X testing for reasons other than a positive family history of mental retardation. Such individuals may include women with POF and individuals with ataxia/tremor. Although there is currently no published literature on the impact of fragile X carrier testing for these individuals, genetic counselors should be aware that the issues and responses to this information may differ significantly from those that have been identified in individuals and families referred for family history of mental retardation. Based on clinical experience, we postulate that areas in which differences might be expected to occur include:

- Unexpected finding—For example: A woman with POF may have previously been very focused on achieving a pregnancy and may have been reassured that if she does become pregnant her risk is no different than any other woman her age. In this circumstance the finding of fragile X syndrome might result in a significant shift in this perception.
- Regret or anger—For example: If testing and the diagnosis of fragile X syndrome occurred after multiple expensive and/or invasive medical procedures or multiple tries at pregnancy, regret or anger may be expressed by the patient/family that testing was not considered sooner in the diagnostic process. For both FXTAS and POF different medical or life choices may have been made if the risk for fragile X syndrome had been known earlier in the evaluation process.
- Implications for family—As noted previously, once the diagnosis of fragile X syndrome has been made there are significant implications

for the extended family. A positive test for fragile X syndrome would be expected to shift the focus from the individual as the "patient" to now include the extended family.

Population Screening

Pregnant and Nonpregnant Women

Although currently not standard of care, offering fragile X carrier screening to pregnant women or women considering pregnancy is becoming more prevalent. Genetic counselors need to be aware of the issues regarding carrier testing in women with no family history of fragile X syndrome and be able to counsel them prior to screening regarding the implications of all possible results. Women found to have expanded allele sizes need counseling and education regarding their particular results, the risks for expansion during pregnancy, reproductive options and implications of their result for other family members. Genetic counselors are likely to receive referrals for women who have had general population screening with results showing premutation or intermediate allele sizes as well as full mutations. Comprehensive education and genetic counseling regarding the implications of these results in regard to family planning as well as risk to other family members is essential.

Women With Intermediate-Sized Alleles

Population carrier screening for fragile X syndrome is likely to detect many women with intermediate-sized alleles. Genetic counseling should emphasize the fact that individuals with intermediate-size alleles are not generally considered to be at risk for clinical manifestations of either pre- or full mutations. Some alleles in this range have been shown to be unstable and to expand in subsequent generations, while others appear stable. The important issue to emphasize is that although guarantees cannot be given for any allele size, to date, no female with fewer than 59 repeats has had a child with a full mutation. Some women with intermediate allele sizes may still request prenatal diagnosis despite this information. Risks and benefits, including cost and potential complications of prenatal diagnosis compared to the negligible risk of having a fetus with a full mutation, need to be discussed at length with clients who are found to have intermediate-size alleles.

PATIENT AND PROFESSIONAL RESOURCES

All clients with a family history of possible or confirmed fragile X syndrome and individuals who are confirmed mutation carriers can be offered patient-oriented resources. Listed below are examples of high quality resources appropriate for clients and families. Health professionals caring for individuals with fragile X syndrome or managing reproductive-related issues in fragile X carriers may benefit from available resources for health professionals (Table III).

SUMMARY

Genetic counseling for fragile X syndrome is challenging because of the complex multigenerational inheritance, variable phenotype, and the implications of these issues for families. Genetic counselors can provide support with an emphasis on anticipatory guidance for families throughout the life cycle—from newborn screening, pediatric evaluations, reproductive counseling, to evaluations of individuals for FXTAS and POF. This important area of genetic counseling will continue to evolve as new information is learned. Additionally, as experts in this area, genetic counselors have an important role in policy development and implementation regarding FMR1 testing.

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