FMR1 and the fragile X syndrome: Human genome epidemiology review

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The fragile X syndrome, an X-linked dominant disorder with reduced penetrance, is one of the most common forms of inherited mental retardation. The cognitive, behavioral, and physical phenotype varies by sex, with males being more severely affected because of the X-linked inheritance of the mutation. The disorder-causing mutation is the amplification of a CGG repeat in the 5' untranslated region of *FMR1* located at Xq27.3. The fragile X CGG repeat has four forms: common (6–40 repeats), intermediate (41–60 repeats), premutation (61–200 repeats), and full mutation (>200–230 repeats). Population-based studies suggest that the prevalence of the full mutation, the disorder-causing form of the repeat, ranges from 1/3,717 to 1/8,918 Caucasian males in the general population. The full mutation is also found in other racial/ethnic populations; however, few population-based studies exist for these populations. No population-based studies exist for the full mutation in a general female population. In contrast, several large, population-based studies exist for the premutation or carrier form of the disorder, with prevalence estimates ranging from 1/246 to 1/468 Caucasian females in the general population. For Caucasian males, the prevalence of the premutation is ~1/1,000. Like the full mutation, little information exists for the premutation in other populations. Although no effective cure or treatment exists for the fragile X syndrome, all persons affected with the syndrome are eligible for early intervention services. The relatively high prevalence of the premutation and full mutation genotypes coupled with technological advances in genetic testing make the fragile X syndrome amenable to screening. The timing as well as benefits and harms associated with the different screening strategies are the subject of current research and discussion. ***Genet Med* 2001:3(5):359–371.**

Key Words: *fragile X syndrome,* FMR1, *FRAXA, mental retardation, epidemiology, review*

FMR1 AND FMRP

FMR1, located at Xq27.3, consists of 17 exons and is approximately 38 kb in size.[1,2] The mRNA is approximately 4.4 kb and contains 1.9 kb of coding sequence. Within the 5' untranslated region (UTR) of *FMR1* is a polymorphic CGG repeat coincident with a rare fragile site on the X chromosome known as FRAXA. FRAXA was a cytogenetic marker instrumental in the identification of patients and, ultimately, the gene itself (see Disease section, below).

FMR1 is highly conserved at the sequence and amino acid level as evidenced by its presence in the human, mouse, *Caenorhabditis elegans, Xenopus laevis, Drosophila melanogaster,* and chicken.[1,3–6] *FMR1* is widely expressed in both human and mouse embryos, with the highest expression located in the brain, testes, ovaries, esophageal epithelium, thymus, eye, and spleen.[7] Extensive alternative splicing of the 3' end of the gene produces different mRNAs and isoforms of the protein, each predicted to have distinct biochemical properties[1] (reviewed in Warren and Sherman).[8] Only a few isoforms are commonly detected in lymphoblasts; however, the existence of other predicted isoforms in different cell types has not been thoroughly investigated.[8]

The fragile X mental retardation protein (FMRP) is the protein product of *FMR1*. FMRP contains two ribonucleoprotein K homology domains (KH domains) and clusters of arginine and glycine residues (RGG boxes), two features commonly associated with RNA-binding proteins.[1,9] FMRP binds in vitro to itself as well as to two fragile X-related autosomal homologs, FXR1P and FXR2P.[10] However, some experiments suggest that these FXR interactions may not exist in vivo.[11–13] Also, recent experiments in rabbit reticulocyte lysate and *X. laevis* oocytes demonstrate that FMRP strongly inhibits translation of various mRNAs at nanomolar concentrations, an effect not seen with its homologs FXR1P and FXR2P.[14]

In addition to its RNA-binding properties, FMRP contains both a nuclear localization signal (NLS) and a nuclear export signal (NES).[15] The current model suggests that oligomerized

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Received: May 2, 2001.

Accepted: June 20, 2001.

FMRP, in conjunction with other proteins, shuttles specific mRNAs from the nucleus to the cytoplasm for translation. Consistent with this model is the RNA-dependent association of FMRP with actively translating polyribosomes.[16,17] Also, other proteins such as nucleolin and nuclear FMRP interacting protein (NUFIP) have been shown to interact with FMRP, possibly modulating mRNA binding to FMRP.[18,19] Furthermore, recent experiments based on the fragile X patient with a Ile to Asn substitution at amino acid position 304 (I304N) suggest that FMRP must oligomerize to properly modulate the transcription of these other mRNAs.[14] Finally, although FMRP has been shown to be primarily a cytoplasmic protein,[20,21] recent immunogold experiments have localized FMRP in the neuronal nucleoplasm and within the nuclear pore,[22] suggesting nuclear export capabilities. Inconsistent with this model, however, is the observation that the *D. melanogaster* homolog, dFMR1, remains in the nucleus even though the conserved NES is present.[6] Despite this inconsistency of biological conservation and significance, identification of the specific, shuttled mRNAs and their respective genes as well as modulating proteins will greatly increase the understanding of FMRP's role in the fragile X syndrome phenotype.

FMR1 CGG REPEAT VARIANTS

The *FMR1* polymorphic CGG repeat located in the 5' UTR of the gene can be categorized in at least four forms based on the size of the repeat: full mutation (>200–230 repeats), premutation (61–200 repeats), intermediate (41–60 repeats), and common (6–40 repeats). Among the general population, the common repeats are usually transmitted from parent to offspring in a stable manner. Intermediate alleles are larger repeats that may or may not be transmitted stably from parent to offspring. Thus, these alleles overlap the boundary between common and premutation alleles.[23] While intermediate alleles may be unstable, very few expand to the disorder-causing mutation in the next generation. Recent evidence, in fact, suggests that intermediate alleles identified among families with the fragile X syndrome are more susceptible to disorder-causing expansions in the next generation compared with intermediate alleles identified in the general population.[23–26] To date, 59 repeats is the smallest size known to expand to the disorder-causing mutation in the next generation within a family with the fragile X syndrome.[23,24] More prospective studies are needed to better quantify the risks of disorder-causing expansions for the purpose of genetic counseling the women identified with intermediate alleles.

Unlike common and intermediate alleles, premutation alleles are unstable when transmitted from parent to offspring and usually expand in the next generation. The size of the repeat expansion is positively correlated with maternal CGG repeat size, with >90 repeats almost always expanding to the full mutation in the next generation.[25] The transition from premutation to full mutation is thought to occur prezygotically.[27,28] In contrast to the female germline, paternal transmission of the full mutation to the offspring is rare. Recent studies suggest that selection against full mutation in sperm is responsible for the differences observed between female and male germline transmissions.[27,29]

The full mutation allele is the form associated with the fragile X syndrome phenotype. All full mutations identified to date are derived from premutation or full mutation alleles from the previous generation. The full mutation allele leads to hypermethylation[30] and deacetylation[31] of *FMR1*, which effectively shuts down transcription of the gene.[32]

Since the molecular characterization of the CGG repeat in 1991,[33] most forms of the repeat have been characterized in all populations studied. This review describes the genotype frequencies of the full mutation and premutation, as both of these are directly related to the expression of the fragile X syndrome phenotype.

FULL MUTATION

Before the cloning of *FMR1*, the inducible FRAXA fragile site was developed as a cytogenetic marker and proved valuable in diagnosing this nonspecific form of X-linked mental retardation.[34] Several studies published in the 1980s using the fragile site as a diagnostic tool estimated that the prevalence of the syndrome was as high as 1 in 1,500 males and 1 in 2,500 females in the general population.[35,36] The cytogenetic test employed by these studies has since been shown to be inaccurate, generating both false-negative and false-positive results.[37]

The cloning of the CGG repeat responsible for the fragile X syndrome provided the opportunity for a more accurate diagnosis using molecular techniques. For this review, published studies (abstracts excluded) that employed DNA-based screening for the fragile X syndrome were considered and described in Table 1. Most studies to date have screened target populations (e.g., special education or mentally retarded populations), yet only a few extrapolated these results to the general population (12/39 studies; Table 1). For these few studies, the general assumption is that all males with the fragile X syndrome will be mentally impaired to some significant degree and will be found only among the targeted population.

Full mutation and males: Caucasian populations of northern European descent

For the Caucasian population, the point estimates for eight studies suggest that the prevalence of the fragile X syndrome ranges from 1 in 3,717[38] to 1 in 8,918[39] males in the general population. The confidence limits, available for only four of these studies, vary widely, with a lower boundary of 1 in 1,333[40] to an upper boundary of 1 in 8,922.[41] Three studies summarized in Table 1 did not report point estimates: two[42,43] provided a range and one[44] provided a lower boundary, all of which fall within the point estimates and confidence intervals reported in the other studies (Table 1).

The problem encountered by all these studies is the extrapolation of the prevalence in the phenotypically-defined target population to that in the general population. Comparison of studies is also complicated because of the differences in the

definitions of the target populations. For example, a study that examined clinically referred persons with mental retardation found a higher prevalence for fragile X in the target population[45] compared with a study that examined persons with language delay.[46] Given the clinical phenotype of the syndrome, however, these differences in the prevalence between the target populations are expected.

To avoid the bias of clinical referrals, the largest studies presented in Table 1[38,40,41,47] screened broadly defined populations with mental retardation in an attempt to capture all males affected with the syndrome. Two of these studies[38,41] broadened the definition to include persons receiving special education services regardless of mental retardation status. Although it is reasonable to assume that these studies have provided the most accurate estimates of the prevalence of the fragile X syndrome in the general population, some speculate that the true prevalence would be higher if 'high functioning' males were not placed in these target populations.[48,49] Only the screening of a large population of consecutive newborns will solve this problem of complete ascertainment.[50]

Full mutation and males: Other populations

Little is known about the prevalence of the fragile X syndrome in other populations. Results of the few available studies suggest the prevalence may in fact differ across populations. For example, a report demonstrates that the majority of fragile X cases reported in Israel are of Tunisian Jewish descent. Given that most of the population of Israel is of Ashkenazi descent, investigators proposed that the prevalence of the fragile X syndrome is higher among Tunisian Jews compared with Caucasians by as much as 10-fold.[51] Also, investigators have noted the lack of large CGG repeats in Native American populations and have suggested a lower prevalence of the syndrome in these populations.[52] Similarly, the Spanish Basque population has been reported to have lower prevalence of males with the fragile X syndrome of pure Basque origin in a mentally retarded population and a lower frequency of large CGG repeats compared with Caucasian populations of northern European descent.[53,54] Finally, investigators in Nova Scotia reported an absence of fragile X cases among their mentally retarded population.[55]

Although intriguing and suggestive, none of these studies in other populations were large and/or population-based. The only two population-based estimates for non-Caucasians were both conducted in African-derived populations. Elbaz et al.[56] examined an Afro-Caribbean population in the French West Indies, and Crawford et al.[38] examined an African American population in metropolitan Atlanta, Georgia, U.S.A. Surprisingly, both studies suggested that the point estimate in these admixed, African-derived populations is approximately 1 in 2,500 in the general population, which is higher than that observed in Caucasian populations (Table 1). Further studies are needed to explore this possible higher general population prevalence as the confidence intervals for both studies overlap with estimates in Caucasian populations of northern European descent.

Full mutation and females

Even less is known about the prevalence of the full mutation among females in the general population. Based on the prevalence of the full mutation among Caucasian males in the general population (~1 in 4,000) and the fact that only females can transmit the full mutation to their offspring, the expected prevalence among females affected with the fragile X syndrome is approximately 1 in 8,000 to 1 in 9,000 in the general population. In a voluntary screening of 8,462 women in Israel who had no family history of mental retardation, Pesso et al.[57] identified one woman with the full mutation. Although the screening scheme described by Pesso et al.[57] is not population-based, the point estimate for females in a Caucasian population is close to that expected.

PREMUTATION

The estimates for premutations available in the literature for males and females in the general population are summarized in Table 2. Because the frequency of premutations is close to zero, 95% confidence intervals were calculated using the equations recommended by Fleiss.[58] For this review, we have chosen to define premutations as 61–200 repeats since these repeats are always unstable and have been found to expand to the full mutation.[23,59] Premutations of smaller size are found in families with the fragile X syndrome; however, these smaller sized premutations found in the general population may or may not be unstable. Thus, the estimate of premutations in Table 2 represent the lower limits of premutation carriers.

Premutation and males

Similar to the data for the prevalence of the full mutation, most of the data on the prevalence of premutations were collected from Caucasian populations. Results from two large, population-based studies and several smaller studies suggest that the prevalence of premutations in males is approximately 1 in 1,000 in the general Caucasian population (Table 2).

Premutation and females

For females, recent large studies have established that the prevalence of premutation carriers is high, with point estimates ranging from 1 in 246 to 1 in 468 in the Caucasian general population.[38,57,60–63] Curiously, the point estimates for the large studies derived from different populations in Israel[57,61] differ, and the confidence intervals for both of these estimates barely overlap (1 in 271 vs. 1 in 468). Although the authors do not comment on the racial/ethnic background of these populations, it is worth noting that population differences that impact prevalence may exist as suggested in the full mutation data.

DISEASE(S)

The hallmark of the fragile X syndrome is mental retardation, which was noted as early as 1943 in a report of a large

Table 1
Prevalence of the fragile X syndrome among males determined by DNA-based techniques (adapted from Warren and Sherman[8])

Country	Target population	No. positive/ No. tested	Estimated prevalence	
			Target population (%)	General population (95% CI)
U.K. (Wessex)[41,121]	SpEd population (ages 5–18 years), unknown etiology	20/3,738	SpEd: 0.5	1/5,530 (1/8,992–1/4,007)
U.S.A. (Atlanta, Georgia)[38,122]	SpEd population (ages 7–10 years), regardless of etiology	Caucasian: 4/1,572	Caucasian SpEd: 0.3	Caucasian: 1/3,717 (1/7,692–1/1,869)
		African-American: 3/752	African-American SpEd: 0.4	African-American: 1/2,545 (1/5,208–1/1,289)
Southwest Netherlands[47]	Schools and institutes for MR, unknown etiology	9/866	Mild MR: 2.0 Moderate/severe MR: 2.4	1/6,045 (1/9,981–1/3,851)
Hellenic population of Greece and Cyprus[40]	Referred clinical population of idiopathic MR	8/611	MR: 1.3	1/4,246 (1/16,440–1/1,333)
Australia (Sydney)[36,37]	Children with MR in SpEd	10/472	MR: 2.1 Mild MR: 0.6 Moderate/severe MR: 5.4	1/4,350[b]
France[123]	Children with DSM-IIIR classification of MR	10/403	MR: 2.5 Mild MR: 1.4 Moderate/severe MR: 3.6	—
U.S.A. (Baltimore, Maryland)[16]	Preschool children referred for language delay	1/379	Language delay: 0.3	—
China (mainland and Hong Kong)[124]	Persons with MR clinically referred or in SpEd	31/902[a]	MR: 3.4	—
India (Delhi)[125]	Clinically referred children with MR, unknown etiology	19/360	MR: 5.3	—
Southern Häme, Finland[126]	Adult males (>16 years) registered in the Southern Häme Care Organization with MR, unknown etiology	6/344	MR: 1.7	1/4,400[c]
Finland[45]	Clinically referred persons with MR	15/305	MR: 4.9	—
U.S.A. (Colorado)[127]	Targeted "high-risk" children (ages 2–18 years with MR, autism, LD, ADHD, family history) in SpEd population	1/299	SpEd: 0.3	—
Japan[128]	Institutionalized persons with MR	8/298	MR: 2.7	—
Indonesia (primarily Javanese)[129]	Schools for mild developmental delay, no cytogenetic abnormality	5/262	Mild MR: 1.9	—
Hellenic population of Greece and Cyprus[130]	Referred clinical population of idiopathic MR	4/257	MR: 1.6 Moderate/severe MR: 2.9 Profound MR: 3.6	—
Brazil[131]	Schools for the mentally disabled	5/256	MR: 2.0 Mild MR: 2.3 Severe MR: 1.6	—
Japan[132]	Males with MR or psychomotor developmental delay, clinically referred	2/256	MR: 0.8	—
Singapore[133]	Children in schools for mild to severe MR, unknown etiology	5/254	MR: 2.0	—
Hong Kong[134]	Persons with mild MR, unknown etiology	1/243	Mild MR: 0.4	—
U.K. (Coventry)[35,37,135]	Children with MR in institutions or SpEd	6/219	MR: 2.7 Mild MR: 1.3 Moderate/severe MR: 6.7	1/4,090[d]

—Continued

family with 11 mentally retarded males and two mildly retarded females.[64] The clinical phenotype associated with the syndrome has since been widened to include a variety of cognitive, physical, and behavioral characteristics (reviewed in Mazzocco).[65] Almost all males with the full mutation exhibit some clinical features of the fragile X syndrome. Also, most

Table 1 *(Continued)*

Country	Target population	No. positive/No. tested	Estimated prevalence	
			Target population (%)	General population (95% CI)
Chile[136]	Children in SpEd with MR of unknown etiology; excluded profound MR	4/214	MR: 1.9	—
Taiwan[137]	Persons with MR of unknown etiology enrolled in SpEd or private day-care centers	4/206	MR: 1.9 Mild MR: 3.8 Moderate/severe MR: 0.8	—
Poland (Warsaw)[42]	Males in institutions or SpEd with MR	6/201	MR: 3.0	1/2,857–1/5,882[e]
Southwest Netherlands[138]	Clinically referred persons with MR and no known family history of fragile X	10/197	MR: 5.1	—
U.S.A. (New Mexico)[139]	Clinically referred persons with MR or behavior disorders, unknown etiology	10/188	MR: 3.7 LD: 1.1 Hyperactivity/AD: 0.5	—
Spain[140]	Persons with MR in SpEd	11/182	MR: 6.0	—
U.K. (Wessex)[59]	SpEd population (ages 5–18 years), unknown etiology	4/180	SpEd: 2.2	1/8,918[f]
Spain[43]	Children in SpEd or clinically referred with MR of unknown etiology; no known family history of MR	5/180	MR: 2.7	1/6,200–1/8,200[g]
Turkey[141]	Clinically referred children with developmental disability	5/166	MR: 3.0	—
Guadeloupe, French West Indies[56]	SpEd population, unknown etiology	11/163	SpEd: 6.7	1/2,359 (1/4,484–1/276)
South Africa[142,143]	Institutionalized males (blacks) with idiopathic MR	9/148	MR: 6.1 Mild MR: 4.2 Severe MR: 7.8	—
U.K.[144]	Institutionalized males with learning disabilities, unknown etiology	1/138	LD: 0.7	—
U.K. (Oxfordshire)[44]	Children in schools for moderate to severe learning difficulties, unknown etiology	4/103	MR: 3.9	1/4,130[h]
Thailand[145]	Children with developmental delay or MR, unknown etiology	5/94	MR: 5.3	—
India (New Delhi)[146,147]	Institutionalized persons with MR with unknown etiology that scored above 40% on a fragile X checklist	9/93	MR: 9.7	—
Spain[55]	Persons in institutions or SpEd with idiopathic MR	8/92	MR: 8.7	—
Brazil[148]	Institutionalized persons with severe MR, unknown etiology	0/83	—	—
Croatia[149]	Children clinically preselected for fragile X DNA analysis on the basis of MR of unknown etiology, a positive family history, and at least on physical and/or behavioral characteristic of the fragile X syndrome	14/81	17.3	—
Mexico[150]	Children clinically referred with MR, unknown etiology	2/53	MR: 3.8	—

SpEd, special education or special schools; MR, mental retardation; LD, learning disability; ADHD, attention-deficit/hyperactivity disorder; AD, attention deficit.
[a]Zhong et al.[124] did not distinguish between males and females in the published manuscript. The numbers presented in Table 1 are derived from personal communication with Dr. Zhong.
[b]Turner et al.[57] provided only a point estimate.
[c]Arvio et al.[126] provided only a range on the basis of past cytogenetic and DNA-based diagnoses.
[d]Morton et al[135] provided only a point estimate.
[e]Mazurczak et al.[42] provided only a range, not a point estimate.
[f]Jacobs et al.[59] provided only a point estimate.
[g]Millan et al.[43] provided a range, not a point estimate. Millan et al.[43] also acknowledged that persons with mild MR might have been missed, so the range could be as high as 1/5,000–1/6,800.
[h]Slaney et al.[44] only provided a lower boundary, not a point estimate.

Table 2

Prevalence of premutation (61–200 repeats) among females and males in the general population (adapted from Warren and Sherman[8])

Country	Target population	No. positive/No. tested		Estimated prevalence (95% CI)	
		Females	Males	Females	Males
Canada (Quebec)[60]	Unselected female blood donors	28/10,624	—	1/379 (1/560–1/267)	—
Israel[61]	Women of reproductive age with no family history of fragile X or MR	39/10,587	—	1/271 (1/377–1/201)	—
Israel[57]	Women of reproductive age with no history of fragile X or MR	18/8,426	—	1/468 (1/766–1/303)	—
Finland[63]	Pregnant women with no known history of fragile X	6/1,477	—	1/246 (1/603–1/119)	—
U.K. (Wessex)[41,121]	SpEd population of boys (ages 5–18 years), unknown etiology	—	2/3,732	—	1/1,866 (1/5,376–1/288)
U.S.A. (Atlanta, Georgia)[38,122]	SpEd population (ages 7–10 years) and their parents	Caucasian: 2/670 African-American: 0/321	Caucasian: 2/2,016 African-American: 0/805	Caucasian: 1/335 (1/1,934–1/84)	Caucasian: 1/1,008 (1/5,814–1/250)
Canada (Ontario) and U.S.A. (Michigan)[151]	Guthrie spots from consecutive male births	—	1/1,000	—	1/1,000 (1/19,134–1/210)
Canada (Winnipeg, Manitoba)[152]	Anonymous, consecutive newborn blood spots	0/735	1/778	—	1/778 (1/14,904–1/163)
U.S.A. (Fairfax, Virginia)[62]	Screening egg donors or pregnant women with no history of MR or LD	3/745*	—	1/248 (1/961–1/93)	—
U.S.A. (Baltimore, Maryland)[153]	Families referred for genetic disorders	1/561	0/416	1/561 (1/10,741–1/118)	—
U.S.A. (Baltimore, Maryland)[154]	Children (ages 5–18 years) with learning or school difficulties, mixed ethnicity	0/341	1/673	—	1/673 (1/12,892–1/141)
Hellenic population of Greece and Cyprus[130]	Referred clinical population of idiopathic MR	0/176	1/257	—	1/257 (1/4,923–1/54)
U.S.A. (Colorado)[127]	Targeted "high-risk" children (ages 2–18 years with MR, autism, LD, ADHD, family history) in SpEd population	0/140	1/299	—	1/299 (1/5,727–1/63)
U.S.A. (Rochester, Minnesota)[155]	Caucasian female blood donors	1/197	0/50	1/197 (1/3,774–1/42)	—

SpEd, special education or special schools; MR, mental retardation; LD, learning disability; ADHD, attention deficit/hyperactivity disorder; AD, attention deficit.
*Spence et al.[62] reported one of the premutations as 60 ± 3 repeats.

affected males do not reproduce, presumably due to the severity of mental retardation. With regard to cognitive function, affected males often exhibit developmental delay very early in childhood. By the age of 3 years, most males will test in the mentally retarded range.[66] Ultimately, almost all males with the fragile X syndrome are mentally retarded, with severity ranging from profound (IQ <20) to mild mental retardation (IQ 50–70), with most being moderately retarded (IQ 40–54) (reviewed in Hagerman).[67] Physically, adult males often have a long narrow face, prominent ears, a prominent jaw, and macroorchidism (reviewed in Hagerman).[67] Other common physical features include a high arched palate, hyperextensible finger joints, double jointed thumbs, single palmar crease, hand calluses, velvet-like skin, flat feet, and mitral valve prolapse (reviewed in Warren and Sherman).[8] Males with the fragile X syndrome also tend to exhibit behavioral features such as hyperactivity, social anxiety, perseverative speech and language, tactile defensiveness, stereotypies (e.g., hand-flapping), and hand biting (reviewed in Hagerman).[67] Autistic-like behavior is also described in these males, with as many as 25% of males with the fragile X syndrome meeting the diagnostic criteria for autism.[68] The association of fragile X with autism, however, is not clear because the proportion of males with the fragile X syndrome meeting the diagnostic criteria for autism seems to diminish with age.[68]

Compared with males, females with the full mutation are often less affected, presumably because of X-inactivation.[69,70] Approximately 30% to 50% of females with the full mutation have an IQ of <70, and 50% to 70% of females with the full mutation have an IQ of <85 e.g.[71] Unlike males with the full mutation, females with the full mutation do not typically have reduced reproductive fitness. Thus, these women are at-risk of transmitting the full mutation to their offspring.

ASSOCIATIONS

Traditionally, persons with premutations were considered clinically normal. However, behavior and cognitive studies of both males and females with the premutation suggest that there may be a mild, measurable phenotype related to this repeat size, although there is also evidence to the contrary.[72–79] At the molecular level, recent experiments using more sensitive techniques than previously employed suggest that males with large premutation alleles (100–199 repeats) have fewer FMRP-positive cells and an elevated *FMR1* mRNA level.[80] Furthermore, experiments also suggest that the decrease of FMRP and the increase of *FMR1* transcript are related to the increasing size of the repeat.[81] These results suggest a mechanism in which the lower translational efficiency presumedly caused by the large repeat is being compensated by the increase in transcription.[80,81] Alternatively, the expanded CGG repeat could lead to a proportionally more open promoter conformation and enhanced transcription.[81,82] What behavioral or cognitive phenotypic effect, if any, these mechanisms have on the individual with the premutation allele is unclear.

The most convincing evidence of a phenotype among female premutation carriers is the ~21% (95% confidence interval: 15% to 27%) who experience premature ovarian failure (POF), compared with 1% in the general population.[83] Whereas the mean age of menopause is 51 years, women with POF experience the cessation of menses before the age of 40. The molecular basis of this phenotype remains unknown. Because full mutation female carriers are not at elevated risk for POF, compared with premutation carriers, the lack of FMRP does not seem to be responsible for POF. The transcript with a large repeat has been suggested to somehow cause the POF phenotype, as this transcript is absent among persons with the full mutation. The exact molecular mechanism of the altered transcript causing POF remains to be elucidated.

INTERACTIONS

No interactions with environmental factors or other genes have been identified for the fragile X syndrome. However, such interactions may be possible as witnessed by the range in clinical severity of fully methylated, full mutation males and females, even among monozygotic twins.[84–86] Among repeat-size or methylation mosaic males and females, variability in IQ can be partially explained by the variability in FMRP levels.[87] However, neither features of *FMR1* nor its gene product FMRP can account for the majority of the variability among fully methylated, full mutation males. For example, size of the methylated full mutation is not correlated with IQ or related to the occurrence other specific characteristics of affected males such as attention-deficit/hyperactivity disorder.[88] Also, while the low but measurable level of FMRP seems to be related to level of development among affected males, it does not seem to be related to the rate of development or to the expression of autism.[89] In fact, the co-occurrence of autistic behavior and the fragile X syndrome more accurately predicts developmental status than does the level of FMRP,[89] possibly suggesting the existence of additional factors involved in the fragile X phenotype.

Interactions affecting CGG repeat stability may also exist. Expansion and resulting size of the premutation are more similar among families than between families, and risk for expansion of premutations is greater among families with affected persons compared with the general population.[23,26] Supportive evidence for an unidentified 'familial' factor can be found in a study that showed full mutation repeat sizes are more similar among siblings than among unrelated patients.[90] Finally, studies examining repeat size variation in sperm suggest that only a small portion of the variance can be explained by factors already identified.[91,92] Identification and description of new factors will lead to a better understanding of which families are at risk of developing a hyperexpanding allele.

LABORATORY TESTS

The Quality Assurance Subcommittee of the American College of Medical Genetics Laboratory Practice Committee has

recently published technical standards and guidelines for fragile X syndrome testing.[24] According to the subcommittee, DNA-based tests that determine the size of the fragile X CGG repeat are considered diagnostic and are 99% sensitive and 100% specific.[24] These DNA-based tests are also applicable for prenatal diagnosis in both amniotic fluid cells and chorionic villus samples (CVS). However, all DNA-based tests for the fragile X syndrome have important caveats that impact the interpretation of the test, most of which are reviewed below. For a more comprehensive checklist, please refer to the standards and guidelines published by the Quality Assurance Subcommittee.[24]

The most popular and accepted method for DNA-based testing for the expanded CGG repeat is the Southern blot. Many different restriction enzymes can be used in combination to determine both expansion (*Eco*RI, *Pst*I, *Bgl*II, *Hin*dIII, *Bcl*I) and methylation (*Sac*II, *Bss*HII, *Eag*I, *Bst*ZI) status for an individual (for a review, see Warren and Sherman, and Murray et al.)[8,93] Methylation status is particularly useful for distinguishing between borderline premutation and full mutation alleles (200–230 repeats).[24] Methylation sensitive enzymes can also describe the degree of methylation of the full mutation allele for both males and females as well as the X-inactivation pattern for females. However, neither of these measures can be used to predict the degree of mental retardation status for either sex.[24] The main disadvantage of the Southern blot is that it requires a large amount of DNA and is laborious, both of which are features that prevent the rapid and inexpensive screening of large populations. Because of these limitations, other diagnostic tests based on DNA and protein properties of the fragile X syndrome have been developed for fragile X screening.

New DNA diagnostic tests have concentrated on the use of the polymerase chain reaction (PCR). Many different PCR protocols have been developed for the fragile X CGG repeat, with different degrees of amplification abilities and sizing accuracies. Regardless of the variations in protocol, compared with Southern blots, the PCR test is inexpensive, automated, and fast. Also, PCR can be performed on very small amounts of DNA, making collection of the samples relatively painless and convenient for the patients. The disadvantage of PCR is that the test results may not be straightforward for several reasons. The amplification of large repeats is difficult, especially in the presence of a second, smaller repeat. For many PCR protocols, the DNA fragment with the expanded repeat does not amplify. This is especially problematic for females and persons with repeat-size mosaicism who could appear to have a single, normal repeat size. To avoid these false negatives, many screening programs follow-up by Southern blot any sample that fails to amplify by PCR and any female who appears to be homozygous. This strategy could potentially produce a false-negative result for persons who are normal/full mutation mosaic; however, few data exist to suggest that this occurs frequently.

Most DNA-based methods can distinguish between premutations and full mutations. Because of ethical issues in identifying asymptomatic carriers, some proposed screening strategies are designed to identify only affected persons. Also, affected persons with point mutations and deletions would not be identified because the sequencing of *FMR1* is not routinely practiced for screening strategies.[94] For the fragile X syndrome, affection status depends not only on the expansion of the repeat, but the subsequent lack of FMRP as well. The development of antibodies against FMRP has made screening possible on the basis of affection status only.[9,21] In this protein-based assay, the percentage of FMRP detected in lymphocytes from blood smears is used to determine affection status.[95–97] Typically, fewer than 40% of the lymphocytes from males with the fragile X syndrome have detectable amounts of FMRP.[96] This protein-based test has recently been adapted for hair root[98,99] and prenatal[100,101] samples. Although promising, this technique cannot accurately identify affected females.[87]

POPULATION TESTING

Syndrome screening

No large, routine programs exist to screen for the fragile X syndrome. As a result, most cases are diagnosed through a referral for testing. In 1994, a working group for the American College of Medical Genetics published guidelines in making referrals for fragile X testing.[102] These include testing any person with unexplained mental retardation, developmental delay, or autism, especially if physical or behavioral characteristics commonly associated with the fragile X syndrome are evident. The working group also recommends testing on the basis of a family history of unexplained mental retardation. On the basis of these recommendations, 1% to 2% of samples referred for molecular testing for the fragile X syndrome are actually positive for the syndrome.[103–105]

Beyond the referral system, investigators in the United Kingdom, United States, and Netherlands have employed active screening systems geared toward school-aged children with mental retardation or other learning difficulties, regardless of family history (Table 1).[38,41,47] The decision for fragile X testing among these nonclinically referred populations differed among the studies. For example, the studies in the United Kingdom and Netherlands tested children with mental retardation or learning difficulties of unknown etiology,[41,47] and the study in the United States screened all eligible children with parental consent regardless of etiology.[38] Despite differences in the decision to test, all three studies identified the fragile X syndrome in school-aged children previously undiagnosed.

Identification of new cases of the fragile X syndrome among school-aged children suggests that the syndrome is not being diagnosed through the referral system.[106] Despite the combination of mental retardation and specific physical and behavioral characteristics associated with the fragile X syndrome (see Disease section), the development of clinical checklists designed to identify children for fragile X screening has been hampered by several factors. First, the physical characteristics in affected adult males may not be seen in young affected males.[107] Macroorchidism is also often absent until the onset of puberty in males. Also, some of the facial features, such as the

prominent jaw, may be racial- or ethnic-specific.[108] Second, the degree of mental retardation and developmental delay among affected males varies widely. This variation in cognitive ability greatly affects the timing of diagnosis. Often, males with severe mental retardation are tested for the fragile X syndrome much earlier in childhood compared with males with moderate or mild mental retardation.[109,110]

Carrier screening

In this section, "carrier screening" refers to identifying women with premutation or full mutation alleles because both are at risk of transmitting a full mutation to their offspring in the next generation. The working group for the American College of Medical Genetics currently does not recommend population carrier screening.[102] In the United States, population-based carrier screening programs for the fragile X syndrome targeting women of reproductive age do not exist. The literature contains reports of smaller screening studies based on self-referral or a family history of mental retardation. One such program at the New York State Institute for Basic Research screened over a 3-year period 344 pregnant women with family histories of mental retardation.[111] Among these women, two had full mutations and four had premutations (defined as >55 repeats). Another program at the Genetics & IVF Institute in Fairfax, Virginia, offered fragile X carrier screening to women on a self-pay basis.[62,112] Most women were referred to the clinic because of their advanced reproductive age. From December 1993 through June 1995, 3,345 women were offered testing, and 668 accepted (21%). Most (69%) of these women did not have family histories of mental retardation. Among these women, three premutation carriers (60–199 repeats) were identified.[62] In a third study conducted at the Center for Obstetric Research at the University of Alabama at Birmingham during 1994–1998, 263 (2.1%) of 12,349 pregnant women reported a family history of mental retardation and underwent carrier testing,[113] and no full mutations or premutations were identified.

Unlike the screening studies performed in the United States, investigators in Finland have implemented a large, population-based carrier-screening program. The program implemented by the Kuopio University Hospital in Finland offered an *FMR1* gene test free of charge to all pregnant women seeking prenatal care from July 1995 until December 1996.[63] According to Ryynanen et al.,[63] almost all pregnant women in Finland seek prenatal care and are registered in antenatal clinics during the 6th through 10th weeks of pregnancy because this registration is required for maternity allowance provided by the state. Among women without family histories of the fragile X syndrome, 1,477 (85%) elected genetic testing. Of these women, six were identified as premutation carriers (60–199 repeats) and all six women elected prenatal testing. The program has since screened an additional 1,358 women through December 1997. Six more women with the premutation allele were identified, and all six elected prenatal diagnosis.[114] The program has also expanded to offer an *FMR1* genetic test to pregnant women undergoing invasive prenatal testing for advanced maternal age or history of a trisomy pregnancy. In this expanded program, 241 (80%) of the 302 women offered the test consented, and one woman was identified as a premutation carrier.[114]

As in Finland, carrier testing is also widely employed and accepted in Israel. At least three groups have published results obtained from their large screening programs. Unlike the program in Finland, these programs offer the test on a self-pay basis and rely on either a self-referral or physician referral for testing. In one published report, the Rabin Medical Center screened 10,587 women who were self-referred and had no family histories of mental retardation during 1995–1998.[61] Women identified as carriers were offered prenatal testing free of charge as instructed by the Israeli Ministry of Health. A total of 39 women were identified as premutation carriers (61–200 repeats), and all elected prenatal diagnosis.[61] In a second report, the Genetic Institute of the Tel Aviv Sourasky Medical Center offered an *FMR1* genetic test to 9,660 women during September 1994 through October 1998.[26] A total of 38 premutation carriers (60–200 repeats) were identified, and all of these women consented to prenatal diagnosis.[26] Finally, during January 1994 through March 1999, the Danek-Gertner Institute of Human Genetics screened 8,426 women of reproductive age who had no family histories of the fragile X syndrome or mental retardation.[57] Among these women, 18 were identified as premutation carriers (61–199 repeats), and one was identified as a full mutation carrier (>200 repeats).

Compared with preconceptional or prenatal screening described above, the most employed strategy for fragile X carrier screening is cascade screening of families identified through an affected proband. This method of screening relies heavily on both identification of the index case and communication among relatives. Using this strategy, the Fragile X Program of the Prince of Wales Children's Hospital in New South Wales, Australia, has identified and counseled 195 families with the fragile X syndrome (or X-linked mental retardation with macroorchidism) since the late 1960s.[115] Follow-up of 44 women identified as carriers by DNA testing who became pregnant during 1990–1993 revealed that 34 (77%) elected prenatal testing. Also, follow-up of the reproductive decisions and outcomes of carrier women demonstrated a 10-fold decline in the birth incidence of males affected with the fragile X syndrome, reflecting both a reduction in the birth rate and an increase in termination of affected pregnancies.[115]

Gaps in research related to screening

The Task Force on Newborn Screening suggests that a disease or condition must meet several criteria to be considered for inclusion in newborn screening programs. The fragile X syndrome arguably meets many of these criteria.[116] Specifically, based on its morbidity and prevalence, the fragile X syndrome is an important public health problem. Approximately 99% of cases diagnosed to date are caused by a single, inherited mutation, making the fragile X syndrome particularly amenable to DNA-based testing for an accurate diagnosis.[24] Furthermore, preliminary studies suggest that case finding and carrier

screening may be economically feasible.[117,118] Finally, although few data exist for the United States, data in Finland and Israel (see, Carrier screening section) suggest that the fragile X test is acceptable.

Although testing for the fragile X syndrome may be feasible and economical, it does not meet several other key criteria for screening in the public health arena. One crucial gap in research is the lack of a cure or effective treatment available for persons with the disorder. This research gap, however, will be difficult to fill. While children with the fragile X syndrome are eligible for early invention services, the effectiveness of these services cannot be proven unless infants are diagnosed with the fragile X syndrome soon after birth.[119] A second gap, at least in the United States, is the lack of general consensus for the appropriate time to screen. Newborn screening will identify affected infants that are eligible for early intervention services. However, identification of an affected person will also identify at-risk families. Although these identified families could benefit from genetic counseling, newborn screening is neither ideal nor designed for identifying most at-risk families for the fragile X syndrome in the general population.[120] Finally, many other issues, including educating health-care professionals and the public about the syndrome, assessing the psychological impact of carrier status, and maintaining families' privacy, should be thoroughly researched and adequately resolved before implementation of any type of routine screening for the fragile X syndrome in the general population.

Acknowledgment

This work was supported in part by NIH R01 grant HD29909 (to SLS).

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Appendix

Online resources for the fragile X syndrome

Resource	World Wide Web URL
Support Groups	
The National Fragile X Foundation	http://nfxf.org
FRAXA Research Foundation	http://www.fraxa.org/
Research	
X-Linked Mental Retardation (XLMR)	http://xlmr.interfree.it/home.htm
Educational	
Human Genome Epidemiology Network (HuGE Net)[a]	http://www.cdc.gov/genetics/hugenet/default.htm
National Institute of Child Health and Human Development	http://www.nichd.nih.gov
Gene Clinics	http://www.geneclinics.com/profiles/fragilex/details.html
Carolina Fragile X Project	http://www.fpg.unc.edu/~fx/index.htm
The ARC of the United States	http://www.thearc.org
Genetic Databases	
Online Mendelian Inheritance in Man (OMIM)	http://www.ncbi.nlm.nih.gov/htbin-post/Omim/dispmim?309550
Human Gene Mutation Database	http://archive.uwcm.ac.uk/uwcm/mg/search/129038.html
Policy	
American Academy of Pediatrics	http://www.aap.org/
The American College of Medical Genetics	http://www.faseb.org/genetics/acmg/pol-16.htm
	http://www.faseb.org/genetics/acmg/index.html

[a]This review will be published on the HuGE Net Web site with modifications.