FMR1 premutation carrier frequency in patients undergoing routine population-based carrier screening: Insights into the prevalence of fragile X syndrome, fragile X-associated tremor/ataxia syndrome, and fragile X-associated primary ovarian insufficiency in the United States

Feras M. Hantash, MS, PhD, Dana M. Goos, BS, Beryl Crossley, MD, Ben Anderson, BS, Ke Zhang, PhD, Weimin Sun, PhD, and Charles M. Strom, MD, PhD

Purpose: Fragile X syndrome is caused by expansion and methylation of a CGG tract in the 5′ untranslated region of the *FMR1* gene. The estimated frequency of expanded alleles (≥55 repeats) in the United States is 1:257–1:382, but these estimates were not calculated from unbiased populations. We sought to determine the frequency of fragile X syndrome premutation (55–200 repeats) and full mutation (>200 repeats) alleles in nonselected, unbiased populations undergoing routine carrier screening for other diseases. **Methods:** A previously validated laboratory-developed test using triplet-primed polymerase chain reaction was used to detect premutation and full mutation alleles in an unselected series of 11,759 consecutive cystic fibrosis carrier screening samples and 2011 samples submitted for screening for genetic diseases prevalent among the Ashkenazi Jewish population. **Results:** Premutations were identified in 48 cystic fibrosis screening samples (1:245) and 15 samples (1:134) from the Ashkenazi Jewish population. Adjusted for the ethnic mix of the US population and self-reported ethnicity in our screening population, the estimated female premutation carrier frequency in the United States was 1:178. The calculated frequency of full mutation alleles was 1:3335 overall, and the calculated premutation frequency in males was 1:400. Based on frequency of larger, ≥70 repeat alleles, and reported penetrance, the calculated fragile X-associated tremor and ataxia syndrome, and fragile X-associated primary ovarian insufficiency frequencies is 1:4848 and 1:3560, respectively. **Conclusion:** Our calculated fragile X syndrome carrier rate is higher than previous estimates for the US population and warrants further consideration of population-based carrier screening. ***Genet Med* 2011:13(1): 39–45.**

Key Words: *fragile X syndrome, FMR1, carrier screening, FXTAS, FXPOI, POF, POI*

From the Department of Molecular Genetics, Quest Diagnostics Nichols Institute, San Juan Capistrano, California.

Dr. Feras M. Hantash, Department of Molecular Genetics, Quest Diagnostics Nichols Institute, 33608 Ortega Highway, San Juan Capistrano, CA 92690. E-mail: feras.x.hantash@questdiagnostics.com.

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Fragile X syndrome (FXS, OMIM 300624) is the most common inherited cause of mental retardation, affecting 1:3000–1:4000 males and 1:6000–1:8000 females.[1] It is caused by the expansion of the triplet nucleotide CGG in the 5′-untranslated region of the *FMR1* gene on the X chromosome. Methylation of the expanded CGG tract and the upstream GC-rich promoter sequence leads to silencing of *FMR1* expression. In males, who have one copy of the X chromosome, FXS presents with moderate intellectual dysfunction, language delay, repetitive motions, elongated facies, protruding ears, and, in adulthood, macroorchidism.[2] Affected females have a milder phenotype, which often consists of attention deficit disorder and/or personality disorder. FXS is also the most recognized syndrome to be associated with autism spectrum disorder[3]; 10% of males with FXS have a diagnosis of autism spectrum disorder.

The American College of Medical Genetics defines *FMR1* CGG alleles as follows: normal, 5–44 repeats; intermediate, 45–54; premutation, 55–200 repeats; and full mutations, > 200 repeats.[4,5] Carriers of normal and intermediate zone alleles are phenotypically normal individuals with no FXS syndromes; there is no evidence to date of expansion from normal or intermediate zone alleles into full mutation in a single meiosis. Males with full mutations are affected with FXS. Individuals carrying premutations are not affected with FXS, but premutation alleles can expand during female oocyte meiosis to >200 repeats (full mutations) that are methylated and silenced. Although all sizes of premutation alleles can expand into a full mutation in single female meiosis, larger premutation alleles expand more frequently to full mutations than do smaller ones.[6–9] The smallest premutation to expand to a full mutation in a single meiosis was 56 repeats as recently reported.[10]

Premutation carriers can present with other syndromes. Around one third of male premutation hemizygotes will be affected with fragile X-associated tremor and ataxia syndrome (FXTAS) by the age of 50 years, and presentation frequency increases with advancing age.[11–13] Various studies have shown that the larger the repeat size, the stronger the chance of presenting with FXTAS. However, the penetrance seems to be around 33% for carriers of alleles of ~70 repeats or more.[12,14–17]

Some female premutation carriers will suffer from fragile X-associated primary ovarian insufficiency[18–20] (FXPOI, previously referred to as premature ovarian failure/insufficiency; http://www.fragilex.org/html/menopause.htm). Female premutation carriers show overall higher levels of follicular stimulating hormone than do normal or full mutation carriers, along with decreased levels of anti-Müllerian hormone, Inhibin A, and Inhibin B—all indicators of ovarian decline.[19,21,22] All women

who are premutation carriers will have an increased risk of suffering from FXPOI,[22,23] with evidence of earlier onset of FXPOI in female carriers of mid premutations range of 80–100 repeats than carriers of shorter or longer premutation alleles.[24,25] Penetrance of FXPOI seems to be higher in carriers of mid premutation alleles (18.6%), when compared with lower or longer premutation carriers, 5.9% and 12.5%, respectively.[23]

Previous studies have estimated premutation and full mutation carrier frequencies in various populations. Reported premutation carrier frequencies are between 1:113 and 1:157 in Israel[26,27]; 1:259–1:397 in Quebec, Canada[28,29]; 1:246 in Finland[30]; and 1:436 in the US state of Georgia.[31] Two studies by Cronister et al.[8,32] showed the premutation carrier frequency to be between 1:257 and 1:382 in the US population. An earlier study of prenatal patients of advanced maternal age and anonymized egg donors from the state of Virginia showed the premutation carrier frequency as 1:248.[33] In a population study of premutation and full mutation carrier frequency from Japan, no mutations were identified in 947 individuals,[34] whereas the premutation carrier frequency in Taiwan was 1:1674.[35] Some of these studies might have underestimated or overestimated premutation frequency due to factors such as definition of premutation (55–200 vs. 60–200 repeats); self-pay, which favors inclusion of more affluent patients; and inclusion of patients with a family history of FXS, learning disabilities, mental retardation, and developmental delay, which can bias toward detecting higher frequencies.

We recently described a method, triplet-primed polymerase chain reaction (TrP-PCR) coupled with capillary electrophoresis, that overcomes the technical hurdles to implementation of carrier screening and that has the potential to be used for newborn screening.[36] In our method, primers hybridize randomly across the CGG repeat tract and at the 3′ junction of the CGG tract and downstream sequences. This assay detects all carriers of premutations and full mutations, both male and female, and including mosaic carriers. When combined with the classic PCR method, this approach enabled robust, rapid, and 100% sensitive assessment of *FMR1* status in all individuals.[36] The new method would reduce the number of Southern blots by ~99%, enabling population and newborn screening for fragile X. Other groups also recently published similar methods.[37–39] In this study, we explored the suitability of implementing the new TrP-PCR method as a screening tool for premutations and full mutations. Using anonymized DNA samples from patients undergoing routine carrier screening for other diseases (cystic fibrosis [CF] and diseases more prevalent in Ashkenazi Jews), we determined the frequency of premutations in these populations and used this information to extrapolate the expected frequency of full mutations and the prevalence of FXS, FXTAS, and FXPOI.

MATERIALS AND METHODS

DNA samples

For this study, we used residual anonymized DNA from clinical samples originally submitted for (1) CF carrier screening or (2) screening for diseases more prevalent in Ashkenazi Jews. For patients with CF, we request ethnicity from the ordering physician or patient. We receive this information approximately 60% of the time. We assumed that samples submitted for Ashkenazi Jewish (AJ) testing were from Ashkenazi Jews. The samples were previously extracted using Qiagen Genovision-M96 robotic station model and reagents as recommended by the manufacturer (Qiagen, Valencia, CA). Table 1 lists the samples used in the study, along with a breakdown by self-identified ethnicity when provided. Greater than 99% of samples were from females.

Table 1 Ethnic distribution and *FMR1* premutation frequency in female screening populations tested[a]

Ethnicity	Samples screened, no. (%)	Premutations identified, no.	Frequency
CF screened	11,759 (85.4)	48	1:245
White	2866	17	1:169
Hispanic	1723 (12.5)	6	1:287
African American/black	247 (1.8)	2	1:124
Asian	254 (1.8)	0	N/A
Other ethnicities	674 (4.9)	2	1:337
Not available	5995 (43.5)	21	1:285
Ashkenazi Jewish screened	2011 (14.6)	15	1:134
Total	13,770 (100)	63	1:219

[a]Specimens tested represent residual samples from individuals undergoing carrier screening for (1) cystic fibrosis or (2) diseases common among the Ashkenazi Jewish population.

Triplet-primed PCR

All samples were first analyzed using TrP-PCR to screen for the presence of premutations and/or full mutations, as described previously.[36] In brief, 3 μL of DNA were mixed with 12 μL of PCR mix. The PCR mix contained 1X reaction buffer, $MgCl_2$ at a final concentration of 2 mM, 1 unit of FastStarTaq polymerase (all from Roche Applied Science, Indianapolis, IN), FMR1F, FMR1R, and M13R primers at 0.6 μM each, and the nucleotides dATP, dCTP, dTTP, and 3-deaza-2-deoxy GTP at a final concentration of 0.2 μM each in 1.7X Q solution (Qiagen, Carlsbad, CA), and 6% dimethyl sulfoxide (Sigma, St. Louis, MO). Cycling conditions were as described previously.[36] Aliquots of 2 μL were mixed with Hi-Di Formamide and Map Marker-1000 size standard (Bioventures Inc., Murfreesboro, TN), injected into an ABI 3730 DNA Analyzer, and analyzed using GeneMapper Software (Applied Biosystems, Carlsbad, CA) as described.[36] Samples showing fragments of ~50 or more repeats were further analyzed using the classic fragile X PCR (FX-PCR) mix[36] for accurate sizing of alleles containing 55–100 repeats. Southern blot analysis was performed on one sample that showed evidence of full mutation.

Data analysis

Calculation of premutation carrier frequency of the US population used data from the US Census Bureau for information on ethnic mix of the US population (http://quickfacts.census.gov/qfd/states/00000.html).

To determine full mutation carrier frequency we used Hagerman calculations.[40] Hagerman proposed that direct, unbiased estimates of the frequency of full mutation alleles in a given population, considering reported premutation frequencies, will require screening samples of at least 50,000 individuals. Absent this large number, Hagerman proposed calculating the predicted full mutation frequency in individuals (male or female) based on the premutation frequency in female carriers using this equation:

Predicted full mutation frequency

$$= \text{Premutation frequency in females} \times S \times 0.5$$

where S is the rate of expansion of a premutation into a full mutation and was calculated to be 0.107 (i.e., ~10.7% of premutations expand to full mutations), while 0.5 is the probability of inheriting either allele.

Hagerman also suggested calculating premutation frequency in males using the following equation:

Premutation frequency in males

$$= \text{Premutation frequency in females} \times (1 - S) \times 0.5$$

To estimate the prevalence of FXTAS and FXPOI in the populations tested, we used the frequency of premutations in males or females identified in this study, the reported penetrance of alleles in male and female carriers, and frequency of larger premutation alleles. For FXTAS, the following equation was used:

Estimated prevalence of FXTAS

$$= \text{Premutation frequency in males} \times P_{\text{FXTAS}} \times \text{Frequency of alleles} \geq 70 \text{ repeats}$$

where P_{FXTAS} is penetrance of larger premutation alleles, estimated to be 33%.[14–17]

FXPOI prevalence was estimated using the following equation:

Estimated FXPOI prevalence

$$= \text{Premutation frequency in females} \times P_{\text{FXPOI}} \times \text{Frequency of alleles} \geq 70 \text{ repeats}$$

where P_{FXPOI} is the penetrance of premutation alleles in FXPOI, estimated to be approximately 12–28%[21,23]; we used 20% as close to an average. Although some studies showed evidence of earlier age of onset of FXPOI and higher penetrance of mid-range premutations of 80–100 repeats,[24,25] we chose to use the definition of higher risk alleles of ≥70 repeats in our calculation as defined by Rohr et al.[22] We choose to use ≥70 repeats in our calculations for FXTAS and FXPOI for consistency in defining larger premutation alleles and because several studies examined penetrance of larger premutation alleles in FXTAS and FXPOI.[16,17,22]

Statistical analysis

Differences in carrier frequency between various ethnic groups were compared using Fisher's exact test and two-tailed P values, set to 0.05 to reject the null hypothesis that the premutation carrier frequencies for comparisons are the same.

RESULTS

Premutation carrier frequency in the US population

A total of 13,770 samples were analyzed in this study, including 11,759 from CF carrier screening and 2011 from AJ carrier screening; >99% were from females. Table 1 lists the breakdown of samples by self-identified ethnicity. More than 43% of samples had no ethnicity information, whereas 4.9% listed "other" or "mixed" ethnicities. Self-identified whites accounted for 20.8% of samples, whereas Hispanics accounted for 12.5% of samples. African American and Asian identified samples each accounted for 1.8% of the samples analyzed.

Figure 1A shows an example of results obtained using our screening method. A sample was identified as harboring a nonexpanded allele (in gray-color part of the plot) and an expanded allele (in pink area of the plot). Furthermore, the two disruptions in the stutter fragments shown in the gray area of the figure (red arrows, Fig. 1A), before the normal repeat allele, indicate the number of AGG interruptions in the middle of CGG tract, here shown to be two. The sample was reanalyzed using classic PCR with gender confirmation. As shown in Figure 1B, the sample was confirmed to be from a female, as only the X-chromosome copy of the amelogenin gene was amplified. The sample showed heterozygosity for 29 and 65 repeats and was, thus, categorized as a premutation allele carrier.

Table 1 lists the results from the various self-identified ethnic groups. The premutation allele frequency was highest among samples from African Americans, followed by AJ, white, and Hispanic individuals; no premutations were identified in Asians. Forty-eight premutations were identified from 11,759 females from CF-screened samples. Thus, the overall premutation carrier frequency in this population was 1:245. The CF-screened sample population analyzed may not be fully representative of the US population, because the American College of Medical Genetics and American College of Obstetricians and Gynecologists CF-carrier screening recommendations were mainly for whites and Ashkenazi Jews. However, CF screening tests are offered to other ethnic groups as well. Fifteen premutations were identified from 2011 females of AJ ethnicity, giving a premutation carrier frequency of 1:134. Two mutations were identified from self-identified African American/black patients (1:124), whereas no mutations were detected in 264 Asian samples. However, differences between various groups did not reach statistical significance ($P = > 0.2$, Fisher's exact test with two-tailed P values).

Our results are limited by the fact that ethnic information for a significant proportion of CF-screened samples, 43.5%, was not available, even though ethnicity is requested when patient samples are submitted for CF carrier screening analysis, as this information helps in calculating individual residual risk for conceiving a child with CF. Samples from this unidentified ethnicity population harbored the most premutations, 21 overall. Because this category will include patients who would have been from white, Hispanic, and other ethnicities listed, our data and conclusions are limited by the unavailability of this information as the distribution of premutations alleles would likely affect the final outcome. Furthermore, of the total samples analyzed where ethnic information was available, the number of samples from African American and Asian individuals analyzed was too low to make any definite conclusion regarding frequency of premutations, although the lack of premutations detected in self-identified Asians is consistent with published reports of low frequency in this population.[34,35] A total of 63 premutations were identified from the total of 13,770 samples analyzed, giving a premutation carrier frequency of 1:219. The distribution of the premutation alleles identified from the CF-screened samples is shown in Figure 2; 12 of the 48 CF-screened samples (25%) harbored ≥70 repeats. Only one of the AJ samples harbored a premutation ≥70 repeats (80 repeats).

Using the premutation frequency from each ethnic group determined in our study and using the US Census Bureau and approximate Census numbers (Table 2) for the number and ethnic composition of the US population, we calculated the female premutation carrier frequency based on the contribution of each ethnic group in our study to the total US population. For

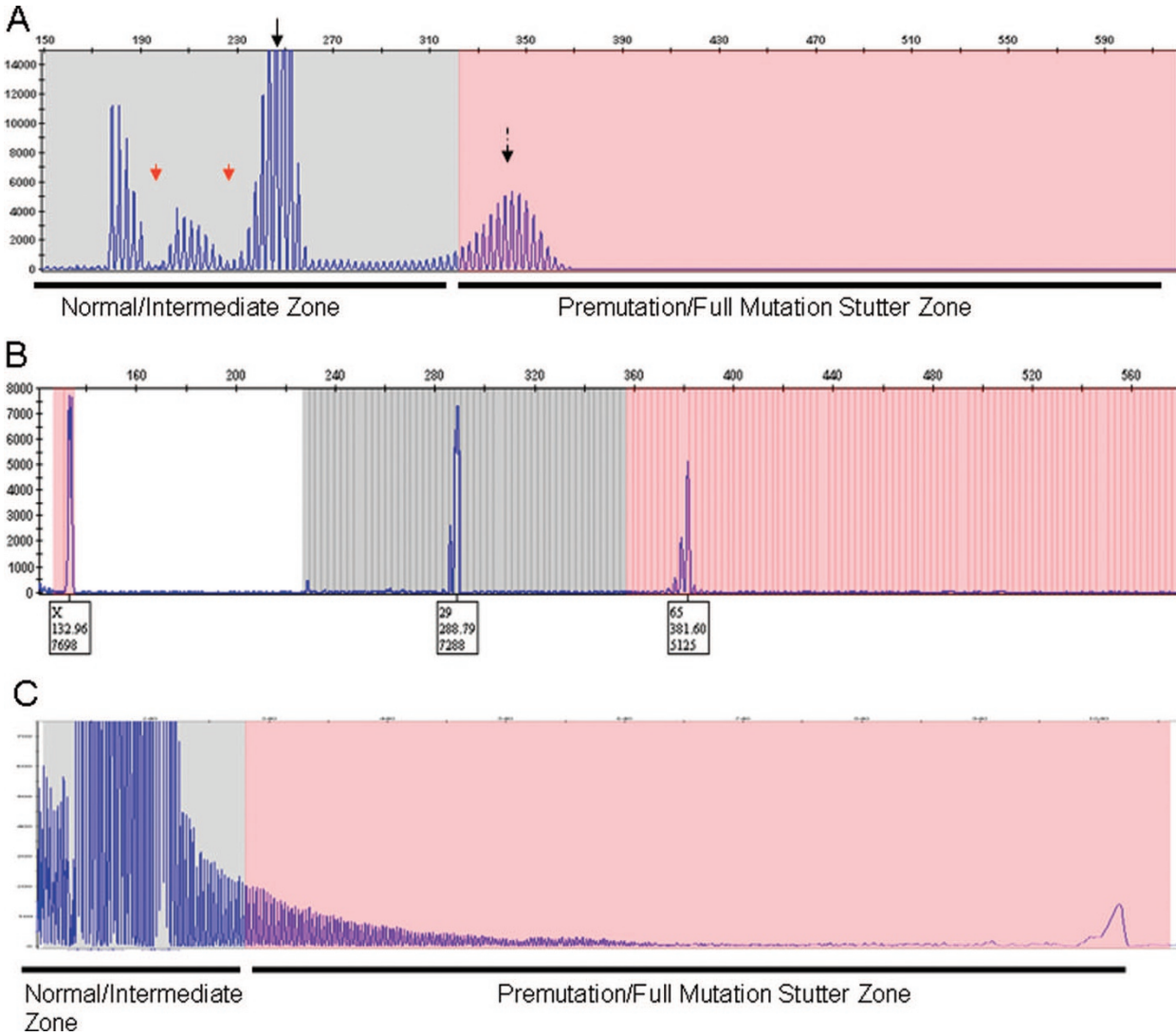

Fig. 1. Detection and confirmation of premutations in samples analyzed. A, An example of a samples analyzed by TrP-PCR[36] showing normal allele in the gray part of the plot (black arrow) and a premutation allele in the pink part of the plot (black dashed arrow). The red arrows refer to depressions in the stutter pattern of fragments, which are likely due to AGG interruptions that hinder PCR amplification. B, Analysis of the same sample in (A) by FX-PCR[41] confirmed gender information as female, as only the X-chromosome copy of the amelogenin gene was amplified, and showing the sample heterozygous for 29 and 65 CGG repeats. C, Detection of full mutation carrier female. One female sample was identified as a likely carrier of a full mutation as "stutter" pattern of fragments was apparent that extended into the premutation and full mutation area of the electropherogram and late migrating peak (arrow) usually detected in a full mutation carrier.[36]

example, when the frequency we obtained for whites from CF-screened population, 1:169, is applied to the number of white females in the US population of 107,560,363 (50.5% of total white males of 195,564,297), the number of white premutation carriers is 578,593. By doing similar calculations from various ethnic groups, the approximate total number of female premutation carriers in the US population is 854,239. By dividing this number by the number of females in the US population of 152,402,657, the extrapolated female premutation carrier frequency is 1:178. We stress that this frequency, although higher than previous studies from the United States,[8,32,33] is only an extrapolation and is limited by the fact that 43.5% of the samples analyzed had no ethnic information available and a limited number of samples from African American/black and Asian ethnicities. However, adjusting the frequency based on ethnic mix of the US population provides better estimation of premutation carriers. The calculated frequency should be further revised once further information is obtained from African Americans, Asians, and other ethnicities.

The CF-screened population included 370 male samples. Premutations were identified in two, including a 61-repeat allele in a white male and a 58-repeat allele in a male with no ethnicity listed. The carrier frequency in this limited data set from males was 1:185.

One female sample, from a white patient, showed evidence of a full mutation as determined by a stutter pattern of ampli-

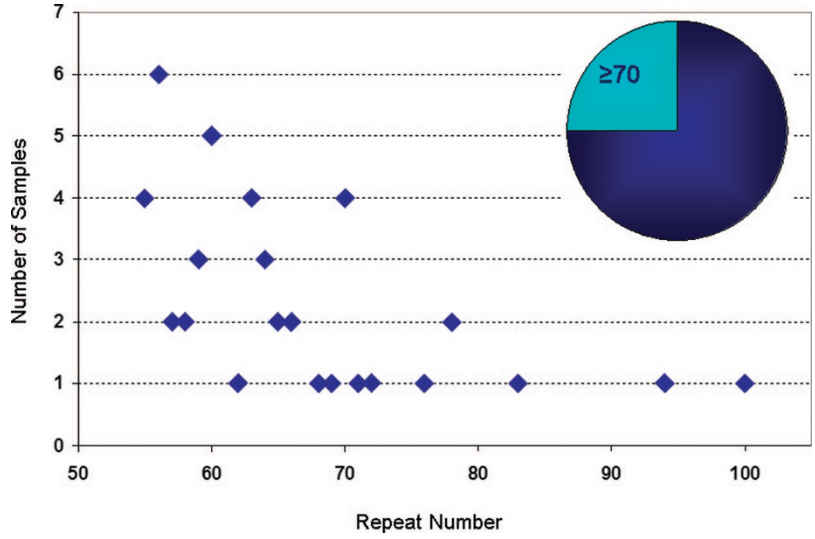

Fig. 2. Premutation allele frequency and distribution of samples analyzed from CF-screened population. Twelve of 48 premutations (25%) detected were ≥70 repeats (inset).

Table 2 Calculated full mutation carrier frequency in the US population

Ethnicity	% of US population[a]	Number in US population	The estimated no. females in the United States[b]	Female premutation carrier frequency	No. female premutation carriers
White	65.6	195,564,297	98,759,970[c]	1:169	584,379
Hispanic/Latino	15.4	47,279,009	23,875,899	1:287	83,191
Black or African American	12.8	39,296,838	19,844,903	1:124	160,040
Asian	4.5	13,815,295	6,976,724	1:1500[d]	21,979
Ashkenazi Jewish	2.2	5,832,000	2,945,160[c]	1:134	4,651
Total		301,787,439[a]	152,402,657		854,239
US female premutation frequency					1:178
US male premutation frequency[e]			= 1/178 × (1−0.107) × 0.5		1:400
Full mutation frequency in the US population (male or female)[e]			= 1/178 × 0.107 × 0.5		1:3335

[a]Information from US census and other online resources (http://quickfacts.census.gov/qfd/states/00000.html, accessed May 31, 2010; US population is 307,006,550; total numbers of US population in table is not identical as other ethnicities are not listed).
[b]Estimates of female numbers assume that females account for 50.5% of the population. Other races are not included. Numbers may not add to actual total female US population due to self-identification of two or more ethnicities.
[c]Whites did not include Ashkenazi Jews, to better estimate overall carrier frequency.
[d]Rough estimate from published reports from Taiwan and Japan.
[e]Calculated using Hagerman equations.[40]

fication and detection of the late migrating fragment on capillary electrophoresis (Fig. 1C). Further analysis by FX-PCR and Southern blot showed the sample to harbor 30/(400–800) repeats, confirming the TrP-PCR finding. The sample showed heterogeneity in DNA methylation (data not shown).

Full mutation frequency in the United States and premutation carrier frequency in US males

Using our calculated female premutation frequency of 1:178 with the Hagerman equations,[40] the calculated full mutation frequency in the US population (males and females) is 1/178 × 0.107 × 0.5 = 1:3335. By ethnic group, the predicted full mutation carrier frequency would be 1:2505 in Ashkenazi Jews, 1:3159 in whites, and 1:5364 in Hispanics.

Using Hagerman equations as well, the calculated premutation frequency in males is 1/178 × (1−0.107) × 0.5 = 1:400. This is different from the observed premutation frequency of 1:185, observed in 370 males from the CF-screened population. However, the number of actual males tested is low, and this could be one reason for the difference between actual versus calculated premutation frequency in males.

FXTAS and FXPOI prevalence

To calculate the expected prevalence of FXTAS, we used the calculated frequency of premutation in males as described earlier (1:400). Taking the calculated premutation frequency in males, the calculated prevalence of FXTAS in the US-screened population is 1/400 × 0.33 × 0.25 = 1:4848 males.

There are different ways to estimate frequency of FXPOI in the general US population. One can calculate FXPOI frequency based solely on the premutation frequency and average penetrance of 20%, which would lead to FXPOI prevalence of 1/178 × 0.2 = 1:890 females. If we calculate based on penetrance of each repeat category as defined by Sullivan et al.,[23] then FXPOI in shorter premutation carriers (as defined by Sullivan et al. of 59–79 repeats) would be 1/178 × 0.059 × 0.729 = 1:4138, where penetrance is 5.9% for carriers of these allele lengths,[23] and the frequency of these alleles from the CF-screened population is 35/48 = 0.729. Similarly, if we calculate the frequency in women with repeats of 80–100 as conveying the highest risk for early onset of FXPOI, only three carriers (3/48, 6.25%) were identified in our study from the CF screened population (Fig. 2). Therefore, the FXPOI frequency would be 1/178 × 0.186 × 0.0625 = 1:14,240 women, where 0.186 represents the penetrance of FXPOI in carriers of this repeat length.[23] We used the penetrance of 20% of larger premutation alleles and frequency of higher risk alleles ≥70 repeats, as classified by Rohr et al.[22] using anti-Müllerian hormone as indicative of early ovarian decline, in our calculations as described in the Materials and Methods section. Using this information, the calculated prevalence of FXPOI in females is 1/178 × 0.2 × 0.25 = 1:3560 females.

DISCUSSION

FXS is a devastating disease for families. The diagnosis of FXS occurs typically around 3 years of age.[42,43] By that time, another affected sibling might have been born. Studies have shown that virtually all families with affected individuals would have taken advantage of population-based carrier screening and prenatal diagnosis had it been available to them.[44,45]

Because FXS is an X-linked disorder, all premutation carrier females are at risk of having affected offspring. Our data demonstrate that if population-based carrier detection for FXS were introduced into the United States, ~1:178 women (extrapolated frequency from this study) or ~1:250 women (this study of CF-screened population and other studies[32,33]) would be determined to be at risk for conceiving children with FXS or related syndromes. This number is much higher than for the three genetic diseases for which American College of Obstetricians and Gynecologists has recommended population-based carrier detection: CF (1:625 at-risk white and AJ couples), Tay-Sachs Disease (1:900 AJ couples), and Canavan Disease (1:4000 AJ couples). The recent review of studies for population and newborn screening for FXS by Hill et al.[46] showed support from health professionals and families of patients with FXS for preconception and prenatal screening, whereas no clear conclusions were drawn regarding newborn screening.

Before the advent of PCR-based techniques for FXS carrier screening, the debate about the appropriateness of recommending population-based carrier screening was purely theoretical, because any method developed allowed reliable screening of males only, as heterozygote carrier females were not reliably detected. Now that their technical barriers have been eliminated,[36,38,39] discussion and pilot studies can begin to address the myriad issues that must be and beginning before undertaking population-based carrier screening and newborn screening for FXS considered.[46,47]

By frequency alone, FXS qualifies for population-based carrier screening and prenatal diagnosis of pregnant, FXS-carrier mothers. However, the complexity of the variable presentation in premutation carriers and full mutation female carriers, as well as the various syndromes associated with *FMR1* repeat expansion (FXS, FXTAS, and FXPOI), could complicate reaching a conclusion to implement screening.

This study demonstrated that the TrP-PCR method used as a carrier screening method for FXS is fast and robust and reduces the burden of performing Southern blot analyses. Even using an expanded analysis of TrP-PCR samples with >50 repeats, the total number of samples analyzed by classic PCR was 219 out of 13,370 samples processed, representing ~1.6% of samples analyzed. This clearly shows the feasibility of using TrP-PCR as a first-line screening tool as only <0.1% of samples (i.e., one sample) required Southern blot analysis. If the approach of combining TrP-PCR and FX-PCR is implemented, and all premutations and full mutations are verified and screened by Southern blot, then 65 (64 premutations and 1 full mutation) would have required Southern blot analysis in our sample population; this equates to 0.5%, which is in line with our previous prediction.[36] Therefore, it is feasible to perform population-based carrier screening using TrP-PCR as a first-line or combined TrP-PCR + FX-PCR. TrP-PCR as a first-line test has the advantage of not missing carrier females, whereas FX-PCR as a first-line test would require TrP-PCR follow-up of 25% of samples to identify female carriers.[36]

In conclusion, we showed in this study the feasibility of TrP-PCR as a first-tier test for FXS carrier screening and demonstrated that the overall calculated premutation carrier frequency of FXS is 1:178. The frequency we report in this study from various ethnic groups warrants consideration of population-based carrier screening for FXS, when ethical and social concerns can adequately be addressed.

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