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Why do fragile X carrier frequencies differ between Asian and non-Asian populations?

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Asian and non-Asian populations have been reported to differ substantially in the distribution of fragile X alleles into the normal (< 55 CGG repeats), premutation (55–199 CGG repeats), and full-mutation (> 199 CGG repeats) size classes. Our statistical analyses of data from published general-population studies confirm that Asian populations have markedly lower frequencies of premutation alleles, reminiscent of earlier findings for expanded alleles at the Huntington's Disease locus. To examine historical and contemporary factors that may have shaped and now sustain allele-frequency differences at the fragile X locus, we develop a population-genetic/epigenetic model, and apply it to these published data. We find that founder-haplotype effects likely contribute to observed frequency differences via substantially lower mutation rates in Asian populations. By contrast, any premutation frequency differences present in founder populations would have disappeared in the several millennia since initial establishment of these groups. Differences in the reproductive fitness of female premutation carriers arising from fragile X primary ovarian insufficiency (FXPOI) and from differences in mean maternal age may also contribute to global variation in carrier frequencies.

Key words: fragile X (*FMR1*) mutation rates, population epigenetics, fragile X-associated tremor ataxia syndrome (FXTAS), fragile X primary ovarian insufficiency (FXPOI), advanced maternal age

INTRODUCTION

Several reports indicate differences among populations in the distribution of fragile X alleles into the normal, premutation and full-mutation size classes (see, for example, Arinami et al., 1993; Barros-Núñez et al., 2008; Hofstee et al., 1994; Nanba et al., 1995; Otsuka et al., 2010; Peprah et al. 2010). Asian and non-Asian populations, in particular, have been reported to exhibit substantial differences, with markedly lower frequencies of premutation- and full-mutation carriers observed in Asian populations (Arinami et al., 1993; Hofstee et al., 1994; Nanba et al., 1995; Otsuka et al., 2010). Here we assess statistical evidence for frequency differences between these groups, using data from several published, general-population studies. We then identify and investigate factors that may establish and sustain these differences, using a new population-genetic/epigenetic model.

Earlier models were constructed to estimate the values of parameters that shape allele and genotype frequencies at *FMR1* (Vogel, 1984; Ashley and Sherman, 1995; Morris et al., 1995; Morton and Macpherson, 1992; Kolehmainen, 1994; Pembrey et al., 1985; Sherman et al., 1985; Sved and Laird, 1990). All of these models provide opportunities to infer values for key parameters, including mutation rates and fitness values. The application of these models has been limited, however, by a paucity of relevant and reliable epidemiologic data. The recent publication of several general-population studies of *FMR1* carrier frequencies in Asian and non-Asian populations offers valuable new data, and encouraged us to revisit these issues.

The model we develop here builds upon an earlier genetic-epigenetic model by Sved and Laird (1990), and considers genotypes that represent all possible combinations of normal, premutation, and full-mutation alleles in transcriptionally active and silent forms (Fig. 1). Under our new model, it is possible to examine individually the impact of each fitness, mutation-rate, and starting-frequency parameter on contemporary frequencies of fragile X premutation carriers in Asian and non-Asian popu-

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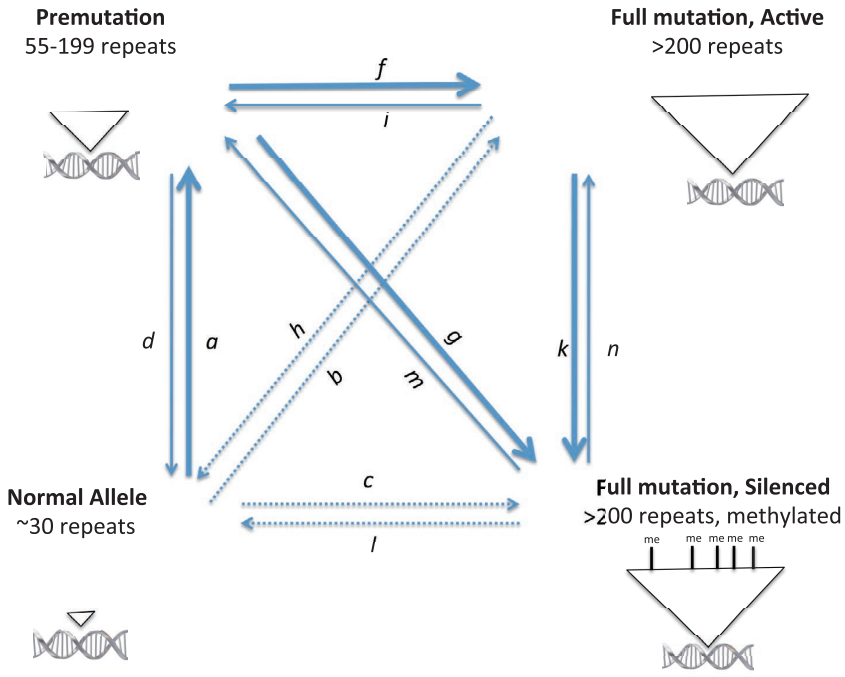

Fig. 1. Schematic of the mutation matrix for normal and expanded alleles at the fragile X locus, *FMR1*. Alleles are classified as: normal (54 or fewer CGG repeats), premutation (55 to 199 CGG repeats), full-mutation/active (200 or more CGG repeats, unmethylated), and full-mutation/silenced (200 or more CGG repeats, methylated). Letters that mark each arrow indicate parameters of mutation and epigenetic transition among these classes. Lines that are bold, standard, and dotted indicate processes that are common, somewhat common, and rare, respectively. To account for the possibility that mutation and epigenetic-transition probabilities differ for egg and sperm lineages, we consider separate parameter values for alleles transmitted via the male (Table 1, middle column) and female (Table 1, right column) germ lines.

lations.

Genetic and epigenetic factors interact to determine the risk of fragile X syndrome and associated phenotypes, suggesting that both may shape allele frequencies at *FMR1*. Genetic variation at *FMR1* includes differences in the numbers of CGG repeats, and in the abundance and placement of the AGG "anchors" that enhance allelic stability (Eichler et al., 1995; Kunst and Warren, 1994). Normal-size alleles expand with low probability-per-transmission to premutation alleles, which in turn expand with higher probability to full-mutation alleles. Among alleles in the normal-size class, only those in the 45-to-54 repeat range, which are sometimes termed "intermediate alleles", have been demonstrated to transition directly into the premutation class. For simplicity, we here use the term "normal alleles" to refer to all those with 54 or fewer repeats. We use the term "normal-to-premutation rate" (Fig. 1) to indicate the overall rate at which premutation alleles arise anew (Genereux and Laird, 2013) from alleles in the normal-size range.

Both premutation and full-mutation alleles can confer disease phenotypes, some of which reduce reproductive fitness. Premutation alleles can lead to two known syndromes: fragile X-associated primary ovarian insufficiency (FXPOI [MIM 311360]) (Cronister et al., 1991), and fragile X-associated tremor ataxia syndrome (FXTAS [MIM 300623]) (Hagerman et al., 2001; Jacquemont et al., 2004). FXTAS, characterized by tremor and ataxia, can occur in male and female premutation carriers, and in individuals with transcriptionally active, full-mutation alleles (Loesch et al., 2012). FXTAS arises after the typical age of reproduction, and is therefore unlikely to impose selective pressure at *FMR1*. In contrast, FXPOI limits the duration of fertility in some female carriers, suggesting that it may reduce fitness, especially in populations where mean maternal age is high.

Epigenetic modification can produce a wide range of phenotypes in individuals with full-mutation alleles. Full-mutation alleles that are epigenetically silenced by DNA methylation and by associated chromatin changes can produce fragile X syndrome in both males and females. Males with full-mutation alleles that are silenced in most or all of their cells have very low levels of the *FMR1* protein, FMRP, and experience moderate to severe cognitive impairment. Males with transcriptionally silenced full-mutation alleles are fertile but, likely due to social factors, rarely have children. Males with unmethylated full-mutation alleles do not have fragile X

syndrome, but may be at risk of FXTAS (Loesch et al., 2012). In females who have a full-mutation allele, random X-inactivation yields phenotypes that range from normal to strongly affected, with disparate reproductive implications (Tuckerman et al., 1985). Thus, epigenetic modification at *FMR1* works in concert with genetic variation to modulate phenotype, with consequences for fitness, and for the frequencies of relevant alleles.

Here, we present our population-genetic/epigenetic model, and use it to identify parameters key in determining population-specific frequencies of females who carry an *FMR1* premutation allele. Applying our model to published data, we find that the lower premutation carrier frequencies reported in Asian as compared to non-Asian populations are most likely attributable to founder-haplotype effects that confer differences in mutation rates, rather than to founder-premutation effects. Selection, too, likely plays a role: we find that one premutation phenotype, FXPOI, may be driving further divergence in population-specific carrier frequencies. Especially large frequency changes are predicted under our model for populations undergoing rapid increases in mean maternal age.

MATERIALS AND METHODS

Construction of a population-genetic/epigenetic model for the fragile X locus, *FMR1* We designed our model to calculate equilibrium allele and genotype frequencies as functions of fitness values, mutation rates, and epigenetic-transition probabilities. Our model tracks four classes of alleles: normal, premutation, full mutation/active, and full mutation/silenced, with rates for forward and back mutation and epigenetic events parameterized as shown in Fig. 1. The distinction between the two full-mutation classes is defined by the presence or absence of dense DNA methylation at the *FMR1* promoter (Pieretti et al., 1991; Stöger et al., 1997, 2011).

In our model, grey-zone alleles with 45 to 54 CGG repeats are considered as part of the normal-allele class, reflecting their very low ---perhaps zero ---probability of expanding directly into the full-mutation class (Cronister et al., 2008; Nolin et al., 2011). Alleles with fewer than 45 repeats are also subject to expansion at very low rates, but have not been observed to expand directly to the premutation class (Cronister et al., 2008; Nolin et al., 2011).

Males, who have a single X chromosome and thus are haploid for *FMR1,* are of four possible genotypes: Normal, Premutation, Full-mutation active, and Full-mutation silenced. Females, who possess two X chromosomes and therefore are diploid for *FMR1,* have ten possible genotypes. Several of the female genotypes that are possible in theory have not yet been reported in either family-based or general-population-based studies. These genotypes are nevertheless considered here, permitting construction of a general model that can be adapted to investigate other diseases that involve premutation and epigenetic components. Similarly, some of the transitions indicated in Fig. 1 have not been observed to occur. These include, for example, single-step expansion from normal to full-mutation alleles. Such events are parameterized here to permit construction of a general model that readily can be adapted to accommodate new data, or to investigate existing data for other loci that share properties with *FMR1.*

Our model tracks mutation, selection, and resulting genotype and allele frequencies across four steps: gamete production, germ-line mutation, mating, and selection. For most calculations, we assume infinite population size. For a few calculations, we implement a finite-population version of the model to investigate how historically small population sizes impact predicted outcomes. This version differs from the main model in that only a subset of the gametes produced is chosen at random to give rise to an individual in the next generation. Both models track allele frequencies over discrete generations. We use computer simulations, as the variables are too numerous to permit analytic solution. All modeling was done in R (R Core Development Team, 2012).

Components of model

(*i*) *Gamete production*

At the start of each generation, male and female individuals contribute gametes to a gene pool. The largest study to date of females with premutation and full-mutation alleles reports that there is no evidence of segregation distortion for normal as compared to expanded *FMR1* alleles (Nolin et al., 2011), a finding that contrasts with some earlier (Pesso et al., 2000) and appreciably smaller (Webb et al., 1986) studies. The present version of our model assumes that alleles in the female germline are transmitted without segregation distortion; our model is flexible, however, and can be modified to consider alternate assumptions for modeling other diseases, and to accommodate any future data indicating that segregation distortion does occur at *FMR1* or other loci.

(*ii*) *Germ-line mutation*

Gametes are subjected to mutation and epigenetic change under the phenomena schematized in Fig. 1. Unless otherwise stated, our calculations were conducted using mutation and epigenetic-transition probabilities given in Table 1, with separate values for male and female germ lines. Germ-line mutation was implemented by multiplying the fraction of alleles that are of a given type by the rate at which alleles of that type transition to each of the other three classes.

Males with full-mutation *FMR1* alleles have been reported to have sperm that carry only premutation alleles (Reyniers et al., 1993; Rousseau et al., 1994), likely

Table 1. Mutation and epigenetic-transition rates assumed for the fragile X locus in male and female germlines

Parameter		Mutation Rates for Male Germline	Mutation Rates for Female Germline
a	normal to premutation	Inferred under model	Inferred under model
b	normal to active full mutation	0.0001	0.0001
c	normal to silenced	0	0.0001
d	premutation to normal	0.01 a	0.01 a
f	premutation to active full mutation	0	0.011
g	premutation to silenced	0	0.099
h	active full mutation to normal	0.001	0.001
i	active full mutation to premutation	0.99	0.01
k	active full mutation to silenced	0	0.95
l	silenced to normal	0	0.001
m	silenced to premutation	0.999	0.02
n	silenced to active full mutation	0	0.01

Legend to Table 1 is in Supplementary Materials

due to selection favoring spermatogonia with smaller alleles (Reyniers et al., 1993). Premutation alleles express *FMR1* in spermatogonia as they do in most other tissues; it is not clear whether these premutation alleles arise from genetic contraction or recombination in the germ line, or from positive selection acting on a genetically mosaic population of cells early in spermatogonial development. For simplicity, we model this phenomenon as a mutational process, whereby full-mutation, silenced *FMR1* alleles always or nearly always transition to premutation alleles during spermatogenesis. Thus, the rate, m, of silenced-full-mutation-to-premutation events occurring in the male germ line is set to 0.99 (Table 1, column 1).

(*iii*) *Mating*

Gametes are paired at random following possible mutation. In the infinite-population model, new genotype frequencies are calculated as the product of the fractions of male and female gametes that carry various alleles. In the finite-population model, only a finite number of gametes contributed by males and by females are chosen and paired at random to contribute to the next generation.

(*iv*) *Selection*

We model selection as the overall set of processes, including survival, fertility, and behavioral factors, that modulate whether or not an individual finds a mate and contributes viable gametes to the gene pool. To implement selection under the infinite-population model, we multiply the fraction of newly produced individuals of a given genotype by the relative-fitness values given in Table 2 (males) and Table 3 (females). Under the finite-population model, individuals persist to the time of reproduction with probabilities that correspond to the relative-fitness values for each genotype. Alternate relative-fitness values are considered in some of the analyses described below.

Table 2. Relative fitness values assumed for males

FMR1 Genotype	Relative Fitness
Normal	1.0
Premutation	1.0
Full Mutation, Active	1.0
Full Mutation, Silenced	0.01

Relative fitness of males with normal alleles: Set to be 1.0.
Relative fitness of males with premutations: Set to be 1.0 because even those males who develop FXTAS typically do not do so until after typical reproductive age (Jacquemont et al., 2004).
Relative fitness of males with full-mutation/active alleles: Set to be 1.0 because, even in the event of FXTAS in a full-mutation male, disease onset is not typically until after reproductive age (see Loesch et al., 2012).
Relative fitness of males with full-mutation/silenced alleles: Males with full-mutation/silenced alleles have fragile X syndrome. Though their fitness is known to be low (Sherman et al., 1984), they do, sometimes, have children (Laird, 1991; Willems et al., 1992).

The R scripts used to perform inferences and run simulations can be downloaded at http://biology.westfield.ma.edu/sites/default/files/basiccode_GenereuxAndLaird2012_0.doc

Model output Previous modeling-based studies of *FMR1* population genetics have focused on the frequency of premutation alleles. By contrast, empirical population-screening studies have typically reported the frequencies of females and/or males who carry a premutation allele. Our model yields values for the frequencies of alleles and carriers. For simplicity, we report here inferred values

Table 3. Relative fitness values assumed for females

FMR1 Genotype	Relative Fitness
Normal/Normal	1.0
Normal/Premutation	0.8 or 1.0
Normal/Full Mutation, Active	1.0
Normal/Full Mutation, Silenced	0.48
Premutation/Premutation	1.0
Premutation/Full Mutation, Active	1.0
Premutation/Full Mutation, Silenced	0.48
Full Mutation, Active/Full Mutation, Active	NA
Full Mutation, Active/Full Mutation, Silenced	NA
Full Mutation, Silenced/Full Mutation, Silenced	NA

Relative fitness for Females with Normal/Normal Genotype: Set to be 1.0.

Relative fitness for Females with Normal/Premutation Genotype: Assumed in most simulations to be either 1.0, as for females with two normal alleles, or 0.8, to account for the possible impact of FXPOI on the timing of menopause and thus family size. Other fitness values were also considered (see Fig. 6 and Supplementary Fig. S2).

Relative fitness for Females with Normal/Full-Mutation/Active Genotype: Assumed to be 1.0, as FXPOI and FXTAS have not been reported to impact these individuals.

Relative fitness for Females with Normal/Full-Mutation/Silenced Genotype: Assumed to be 0.48, following Morton and MacPherson (1992). While these females are at risk of fragile X syndrome, the severity of the phenotype varies widely due to random X-inactivation, with reduced fitness for those with a substantial fragile X phenotype.

Relative fitness for Females with Premutation/Premutation Genotype: Assumed to be 1.0, in the absence of specific information. Females with two premutation alleles are much less common than normal/premutation carriers, so their fitness has little impact on the overall dynamics of the model.

Relative fitness for Females with Premutation/Full-Mutation/Active Genotype: Assumed to be 1.0. Such females are rare, in part because full-mutation alleles are rarely if ever transmitted through the male germ. The fitness of these females therefore has little impact on the overall dynamics of the model.

Relative fitness for Females with Premutation/Full-Mutation/Silenced Genotype: Assumed to be 0.48, following Morton and MacPherson (1992). Such females are rare, in part because full-mutation alleles are rarely if ever transmitted through the male germ. These females would be at risk for both fragile X syndrome and FXPOI, but the severity of these phenotypes would vary broadly due to random X-inactivation, with reduced fitness for those with a substantial fragile X and/or FXPOI phenotype.

Relative fitness for Females with two full-mutation alleles in either silenced or active epigenetic state: Fitness values for these females are not considered in our model. Such females are not predicted to occur, as the full-mutation allele rarely, if ever, is transmitted through the male germline.

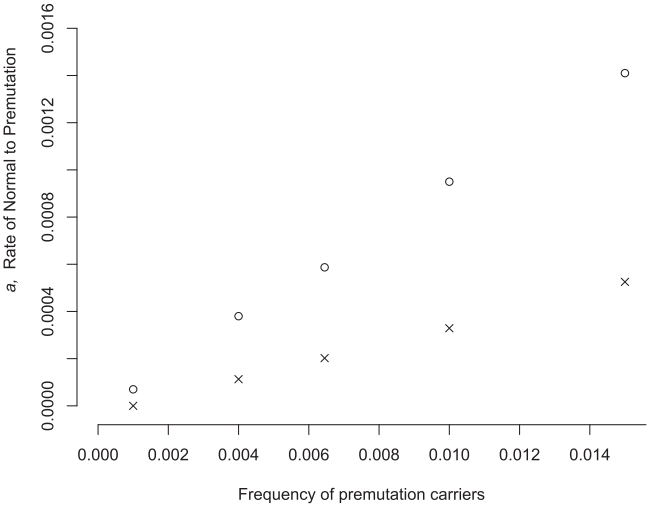

Fig. 2. Inferred rate of normal-to-premutation events required to sustain female premutation carriers at given frequencies. Inferences were made under the assumption of female-carrier fitness of 1.0 (Xs) or 0.8 (circles). Other parameters were set to the values given in Tables 1 and 2.

for the frequencies of female normal/premutation heterozygotes, which we term "carriers". Both infinite- and finite-population models enable us to track allele and genotype frequencies over multiple generations.

Model validation We found that the inferred rate of normal-to-premutation events depends strongly on the equilibrium frequency of *FMR1* premutation carriers (Fig. 2), on the rate of premutation-to-full-mutation events (Supplementary Fig. S1), and on the relative probability of reproduction for female premutation carriers (Supplementary Fig. S2). The first of these rates was estimated by Hagerman (2008) to be ~0.11 per generation. The equilibrium frequency of *FMR1* premutation carriers depends only weakly on the fitness of individuals with fragile X syndrome (Supplementary Fig. S3, a–b). We therefore focused our modeling efforts on the rate of normal-to-premutation events (Fig. 1), and the reproductive fitness of female premutation carriers (Supplementary Fig. S2). Validation of the model is described further in Supplementary Materials.

Compilation and statistical analysis of published data on carrier frequencies Our criteria for including data from a given study were as follows: (i) data were drawn from the general population, rather than from individuals identified through a clinical or family history that was suggestive of cognitive impairment or other *FMR1*-associated phenotypes; and (ii) the total number of individuals assayed was stated specifically. Our analyses here include eight studies that met these criteria: two from the United States (Cronister et al., 2008; Saul et al., 2008), three from Israel (Pesso et al., 2000; Toledano-

Alhadef et al., 2001; Berkenstadt et al., 2007), one from Australia (Metcalfe et al., 2008), one from Japan (Otsuka et al., 2010), and one from Taiwan (Tzeng et al., 2005). Prior to analysis, data were prepared for comparison across studies according to the methods described in Supplementary Materials. Statistical comparisons of carrier frequencies in different populations were made using χ^2 tests, followed by Bonferroni correction for multiple comparisons (Bonferroni, 1936).

RESULTS AND DISCUSSION

***FMR1* premutation carrier frequencies differ significantly between Asian and non-Asian populations** We compiled data from eight published general-population studies to ask whether the frequency of female premutation carriers is variable around the world. These studies used a variety of different criteria to bin *FMR1* alleles into size classes. To accommodate these discordant classifications, we calculated reverse cumulative frequencies across a range of allele sizes, summing frequencies starting with those for the largest allele, down to those for the smallest allele that was considered by any study to exceed the range of normal repeat counts.

The studies we examined reported data for samples from females of reproductive age (Pesso et al., 2000; Toledano-Alhadef et al., 2001; Berkenstadt et al., 2007; Cronister et al., 2008; Metcalfe et al., 2008), from males and females (Otsuka et al., 2010), or from males only (Tzeng et al., 2005; Saul et al., 2008). Because females have two X chromosomes, and males have only one, the finding of one heterozygous female carrier is equivalent, in terms of premutation frequency, to the finding of one male with a normal allele, and one with a premutation allele. To compile and compare data from males with data from females, we divided by two the total number of males sampled, thus standardizing data across studies to obtain the number of pairs of alleles assayed, and rendering male data comparable to data from females. The frequency of premutation alleles was found not to differ significantly between males and females (Supplementary Materials). Using data from males and females, and the common definition of premutation alleles as having between 55 and 199 repeats, the reported or inferred frequencies of female premutation carriers were found to range from 0 to 0.015, with a mean frequency of 0.0060 across all eight studies (Fig. 3; Table 4).

Cumulative distribution curves constructed using these standardized data were similar in shape across the several studies. Data for Asian and non-Asian populations follow qualitatively similar curves, but are almost completely non-overlapping: the distributions for Asian populations are left-shifted relative to the distributions for non-Asian populations, indicating an overall lower mean repeat count for expanded alleles in Asian populations.

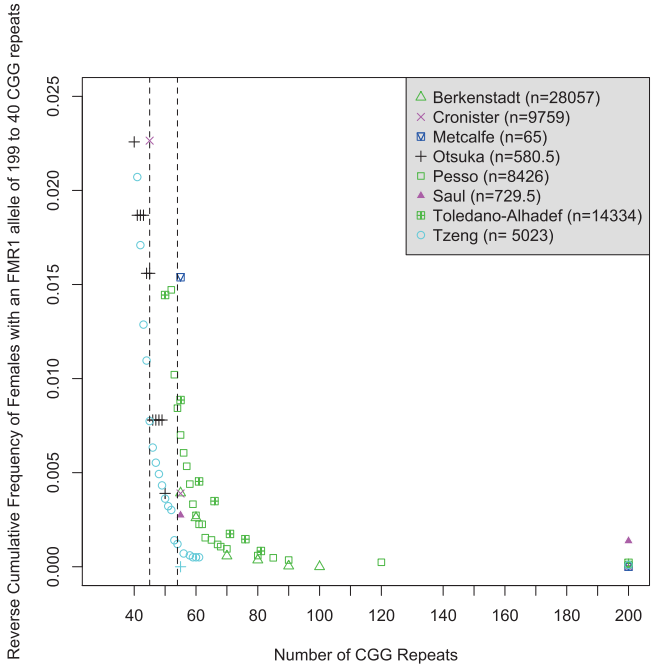

Fig. 3. Inverse cumulative frequencies of female carriers of *FMR1* alleles of various sizes in different populations. Estimates of the frequencies of females with various sizes of *FMR1* alleles were compiled from eight different general-population studies. Inverse cumulative frequencies were plotted according to numbers of CGG repeats. Symbols were assigned as follows: Israel (green), United States (magenta), Japan (black), Taiwan (turquoise), and Australia (blue). Alleles of sizes to the left of the vertical dashed line at 45 repeats are classified by most studies as normal alleles. Alleles of sizes to the right of the vertical line at 54 repeats are classified by most studies as premutation alleles. Alleles of sizes between the two lines have been termed "gray zone" or "intermediate" alleles, and are sometimes considered to belong to the normal class, as they are here. To examine differences among populations in the distribution of alleles into these various size classes, we compiled frequency data for alleles with between 40 and 199 repeats. We calculated cumulative frequencies starting with the largest premutations (199 repeats), and progressed down by size to alleles of 40 repeats, the smallest alleles reported in at least two of these studies. When allele counts were binned into size ranges, data were plotted at the lowest repeat count for that bin. This process yielded the inverse cumulative distributions shown here. The graph also includes data on full-mutation alleles. Alleles with 200 or more repeats were graphed at 200 CGG repeats.

We next tested for statistical significance of these apparent differences in repeat distributions across populations. We asked about heterogeneity in premutation-carrier frequencies among all studies considered (Pesso et al., 2000; Toledano-Alhadef et al., 2001; Tzeng et al., 2005; Berkenstadt et al., 2007; Cronister et al., 2008; Metcalfe et al., 2008; Saul et al., 2008; Otsuka et al., 2010), among studies in non-Asian populations (Pesso et al., 2000; Toledano-Alhadef et al., 2001; Berkenstadt et al., 2007; Cronister et al., 2008; Metcalfe et al., 2008; Saul et al., 2008), among studies in Asian populations (Tzeng et al.,

Table 4. *FMR1* premutation carrier frequencies from eight general-population studies

Study	Location	Units Reported	Number Sampled	Number Premutation Carriers (55-199 Repeats)	Carrier Frequency
Metcalfe et al.	Australia	females	65	1	0.01538
Cronister et al.	US	females	9759	38	0.00389
Saul et al.	US	newborn males	729.5	2	0.00274
US data			10488.5	40	0.00381
Toledano-Alhadef et al.	Israel	females	14334	124	0.00865
Berkenstadt et al.	Israel	females	28057	173	0.00617
Pesso et al.	Israel	females	8426	58	0.00688
Israeli data			50817	355	0.00699
Non-Asian data			61370.5	396	0.00645
Otsuka et al.	Japan	males and females	580.5	0	0.00000
Tzeng et al.	Taiwan	newborn males	5023	6	0.00119
Asian data			5603.5	6	0.00107
Worldwide data			66974	402	0.00600

Table 5. $\chi 2$ tests for heterogeneity in carrier frequencies

Comparison	$\chi 2$ value	p-value	Bonferonni-adjusted p-value
Worldwide (8 studies)	50.27	1.00E-08	5.0E-08
Worldwide, without Asian populations (6 studies)	23.6	2.70E-04	0.0014
Asian Populations (2 studies)	0.69	0.406	1.000
Israel (3 studies)	8.4	0.020	0.100
United States (2 studies)	0.24	0.300	1.000

2005; Otsuka et al., 2010), among studies in Israeli populations (Pesso et al., 2000; Toledano-Alhadef et al., 2001; Berkenstadt et al., 2007), and among studies in U.S. populations (Cronister et al., 2008; Saul et al., 2008).

To assess statistical support for apparent differences in the frequencies of carriers of premutation alleles under the definition of 55 to 199 repeats, we conducted χ^2 tests and applied Bonferroni correction (Bonferroni, 1936) for multiple comparisons. These statistical tests revealed significant heterogeneity among the eight studies in the reported frequencies of premutation carriers (Table 5; adjusted $p = 5.0 \times 10^{-8}$). We infer that the majority of the heterogeneity in the published data exists for non-Asian as compared to Asian populations, as there was no evidence of heterogeneity among studies in Israel (adjusted $p \approx 0.1$), in the United States (adjusted $p = 1.0$), or between studies conducted in Asia (adjusted $p = 1.0$).

Our calculations thus confirm and extend several earlier reports that the frequency of premutation carriers differs for Asian as compared to non-Asian populations (see, e.g., Arinami et al., 1993; Hofstee et al., 1994; Nanba et al., 1995; Otsuka et al., 2010). Our analysis also yielded evidence that there is heterogeneity among non-Asian populations worldwide (adjusted p-value of 0.0014; Table 5), though at a lesser degree than that observed for Asian as compared to non-Asian populations, suggesting the possibility of variation at several geographic scales.

Assessing the factors that may underlie disparate *FMR1* carrier frequencies in Asian and non-Asian populations A key goal in building our model was to assess the potential contributions of mutation rates, founder effects, and carrier fitness in determining equilibrium *FMR1* carrier frequencies, with special emphasis on frequencies in Asian as compared to non-Asian populations. The effects of genetic and epigenetic shifts can be examined under our model by applying various values for the bidirectional genetic and epigenetic transitions schematized in Fig. 1. The effects of selection can be examined using ranges of proposed fitness values. We also explore the multi-generation trajectories of carrier frequencies following possible founder effects by adjusting initial carrier frequencies.

Several studies (see, for example, Hagerman, 2008) have emphasized the role of founder effects in shaping global genetic variation. For disease alleles that arise through mutational expansion, two classes of founder effects are possible. Under the founder-premutation hypothesis, carrier frequencies differ among populations whose founders contributed disparate frequencies of *FMR1* premutation alleles. An extreme form of the founder-premutation hypothesis would hold that a pre-

mutation allele arose just once in human history, and was disseminated, by chance, in variable abundance to different populations. Under the alternative founder-haplotype hypothesis, haplotypes with disparate *FMR1* mutation rates were introduced by the founders of various populations. Differences in the reproductive fitness of female premutation carriers could also lead to divergence of carrier frequencies. Here, we use our model to investigate the potential roles of these factors in determining contemporary carrier frequencies in Asian and non-Asian populations.

The founder-premutation hypothesis does not account for variation in contemporary carrier frequencies either without or with recurrent mutation We first assessed the capacity of founder-premutation effects to account for worldwide variation in *FMR1* allele frequencies in the absence of recurrent mutation. We used our model to investigate the long-term trajectories of carrier frequencies assuming founding-carrier frequencies ranging from 0.0 to 0.1. These calculations were made under our infinite-population model, which seems appropriate in light of global population sizes over the past 100 generations. Even with carrier fitness of 1.0, we find that carrier frequencies would decline to a value near zero within twenty generations in the absence of recurrent mutation, under the assumed founding frequencies (Fig. 4A). This predicted near disappearance of premutation alleles in the absence of recurrent mutation is not consistent with the observation of appreciable frequencies of premutation carriers in most human populations (Table 4; Pesso et al., 2000; Toledano-Alhadef et al., 2001; Tzeng et al., 2005; Berkenstadt et al., 2007; Cronister et al., 2008; Metcalfe et al., 2008; Saul et al., 2008), though perhaps not in all populations (Otsuka et al., 2010).

An alternative to the strict founder-premutation hypothesis is that recurrent mutation works in concert with founder-premutation effects to sustain disparate carrier frequencies, as, for example, calculated here for Asian

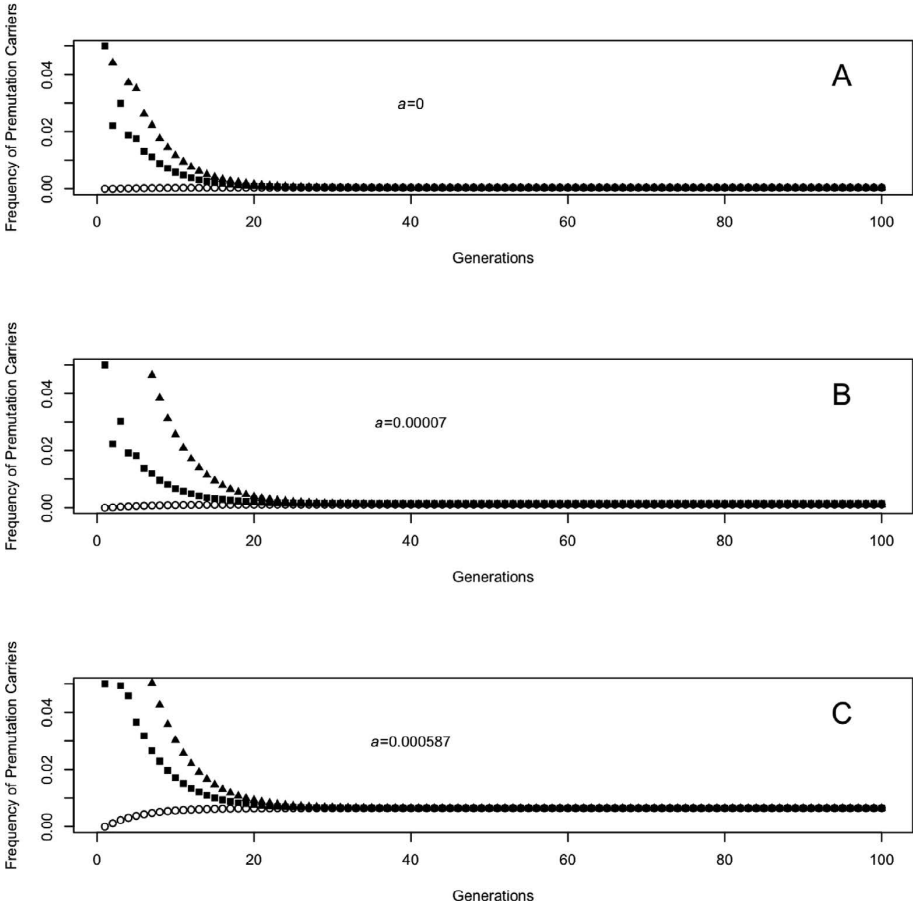

Fig. 4. Trajectory of female premutation-carrier frequencies over 100 generations, given various starting frequencies and assumptions about a, the rate of normal-to-premutation events. Predicted premutation-carrier frequencies are shown for simulations run in the absence (a = 0, A) and presence (a = 0.00007, B; and a = 0.000587, C) of recurrent mutation from normal to premutation alleles. Carrier fitness was assumed to be either 1.0 (A and B) or 0.8 (C). Starting carrier frequencies were assumed to be 0.1 (triangles), 0.05 (squares) or 0.00 (circles). Other parameter values were as in Table 1.

as compared to non-Asian populations. We find, however, that equal rates of mutation, either low (0.00007, Fig. 4B) or high (0.000587, Fig. 4C) result in convergence by generation 30 --- roughly 600 to 900 years --- of initially disparate carrier frequencies, assuming either perfect (Fig. 4B) or imperfect (Fig. 4C) fitness for carriers. This interval of less than a millennium is much briefer than the tens of thousands of years (reviewed in Stanyon et al., 2009) inferred to have passed since the arrival of modern human populations in Europe and in Asia. We conclude that, even in the presence of recurrent mutation occurring at equivalent rates worldwide, the founder-premutation hypothesis cannot account for contemporary global variation in *FMR1* carrier frequencies.

New mutation occurring at disparate rates, as proposed by the founder-haplotype hypothesis, can explain disparate carrier frequencies in Asian and non-Asian populations The inferred normal-to-premutation rate has strong, linear dependence on the observed frequency of female premutation carriers, as noted above (Fig. 2). This finding suggests that different rates of normal-to-premutation, as proposed under the founder-haplotype hypothesis (Richards et al., 1992; Chakravarti, 1992; Jacobs et al., 1993), could be sufficient to yield substantial variation in carrier frequencies. This founder-haplotype hypothesis is also consistent with earlier reports that Asian and non-Asian populations may differ in their distributions of *FMR1* alleles with various numbers and positions of AGG repeats within the CGG-repeat region (see, for example Hirst et al., 1997).

We find that different rates of normal-to-premutation events can, indeed, account for the carrier-frequency differences observed for Asian as compared to non-Asian populations. To sustain the 0.00645 carrier frequency calculated for non-Asian populations, we infer normal-to-premutation rates of 0.000587 and 0.00020, assuming reduced and normal carrier fitness, respectively (Fig. 2; Table 6). To sustain a carrier frequency of 0.001, as estimated for Asian populations, markedly lower mutation rates of 0.00007 and 0 were inferred, assuming carrier fitnesses of 0.8 and 1.0, respectively (Fig. 2; Table 6). Comparable differences in inferred mutation rates hold for all fitness values less than 1.1 (Supplementary Fig. S2).

We were surprised to infer a mutation rate of 0.0 for Asian populations with carrier frequency 0.001 under the assumption of normal fitness for carriers (Table 6). We asked which features of our model could preserve non-zero carrier frequencies in the absence of new mutation. Under our model, we assume that premutation alleles arise at a high rate through new mutation from normal alleles, but also arise at much lower rates (≤ 0.01) through back-mutation from silenced and active full-mutation alleles (Table 1). We find that this low rate of back mutation is sufficient to sustain carrier frequencies at low but non-zero levels: setting this back-mutation rate to zero yields premutation-carrier frequencies that decline to 0 in the absence of new mutation. Such back mutation from full mutation to premutation alleles, perhaps amplified by germ-line selection favoring the transmission of smaller, transcriptionally active alleles, has been found to occur at appreciable rates in the male germline (Reyniers et al., 1993). Back mutation may also occur at low rates in the female germ line (Follette and Laird, 1992). Additional empirical data will reveal the respective contributions of normal-to-full-mutation rates and of back-mutation rates to carrier-frequency differences between Asian and non-Asian populations.

Table 6. Inference of normal-to-premutation rate for Asian and non-Asian populations

Population Group	Female carrier fitness w2 = 0.8	Female carrier fitness w2 = 1.0
Asian	0.00007	0
Non-Asian	0.00058	0.0002

Our inference of disparate rates of normal-to-premutation events is consistent with early suggestions of chromosomal haplotype effects on *FMR1* stability (Richards et al., 1992; Chakravarti, 1992; Jacobs et al., 1993), and with subsequent analyses of the substructure of CGG repeats, which revealed greater stability for alleles with AGG interruptions (Eichler et al., 1995; Kunst and Warren, 1994). Significant differences in the abundance and locations of stabilizing AGG interruptions have been reported for alleles sampled in Japan as compared to alleles sampled in non-Asian populations (Hirst et al., 1997). These unusual alleles, however, are a subset of those sampled in Japan; most of the alleles identified by Hirst et al. (1997) have AGG-interruption patterns shared with non-Asian populations. The many-fold difference in mutation rates that we infer here for Asian as compared to non-Asian populations thus cannot be explained entirely by the presence of these low-frequency alleles of differing repeat structures.

Moderate reductions in carrier fitness due to FXPOI can produce disparate carrier frequencies, even with genetic drift As noted above, primary ovarian insufficiency (FXPOI) can lead to early menopausal features in a subset of female *FMR1* premutation carriers. Early menopause in carriers will affect family size, potentially limiting transmission of the expanded *FMR1* allele. The impacts of FXPOI on the transmission of premutation alleles will be most severe in cultures where reproduction is often delayed until later in life (Fig. 5A), or distributed across many years (Fig. 5B). Comparatively minor impacts will occur in cultures for which births tend to occur earlier in life (Fig. 5C). The high

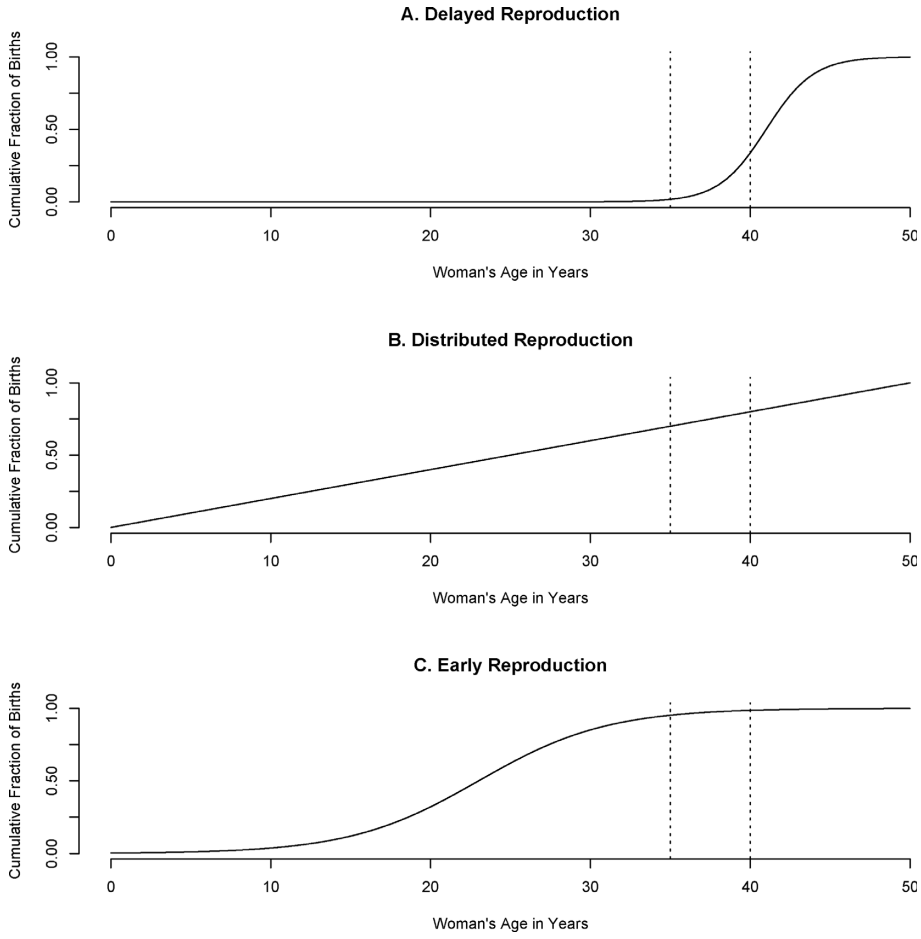

Fig. 5. Schematic of average cumulative fraction of births complete as a function of woman's age. Average cumulative fractions of births complete at a given age are plotted for societies with Delayed Reproduction (A), Distributed Reproduction (B), and Early Reproduction (C). Vertical lines intersect inverse-cumulative-frequency curves to indicate the extent of fertility loss due to menopause occurring at either 35 years (left line) or 40 (right line) years. Loss of fertility due to FXPOI is predicted to be most acute for populations with delayed reproduction (A).

mean maternal age reported for Japan, one of the two Asian populations for which we compiled published data, suggests that reduced fecundity for carriers may contribute to lower carrier frequencies in that population. In turn, carrier frequencies are higher in the United States (Table 4), where mean maternal is appreciably lower. Data on carrier frequencies and mean maternal age are then consistent with the possibility that FXPOI reduces carrier fitness by disfavoring the transmission of expanded *FMR1* alleles, thus reducing their frequency.

Would these predicted reductions in carrier fitness be sufficient to modulate allele frequencies, even with the appreciable impacts of genetic drift anticipated for small populations? To address this question, we used the finite-population version of our model to track allele frequencies in populations of specified size. We assumed a population of n = 8000, a highly conservative estimate for the sizes of most human populations over the past several hundred years, and one which will tend to exacerbate the role of genetic drift.

We first asked whether a difference in carrier fitness in a population of this size could, on its own, explain differences in carrier frequencies observed for Asian as compared to non-Asian populations. We set initial carrier fitness to be 1.0, a value equivalent to that of females with two normal alleles, and asked about the trajectory of allele frequencies as carrier fitness declined to 0.8, a value corresponding to a 20% lower number of children born to carriers relative to normal individuals. With this reduced carrier fitness, carrier frequencies declined within ~10 generations from a starting frequency of 0.012 (Fig. 6A) to a substantially lower mean of 0.004 (Fig. 6B). This frequency, while substantially reduced relative to the scenario in which premutation carriers have fitness equivalent to that of normal females, was not as low as the 0.001 we calculated above for Asian populations (Table 4), and so does not fully account for observed differences. Indeed, even under our infinite-population

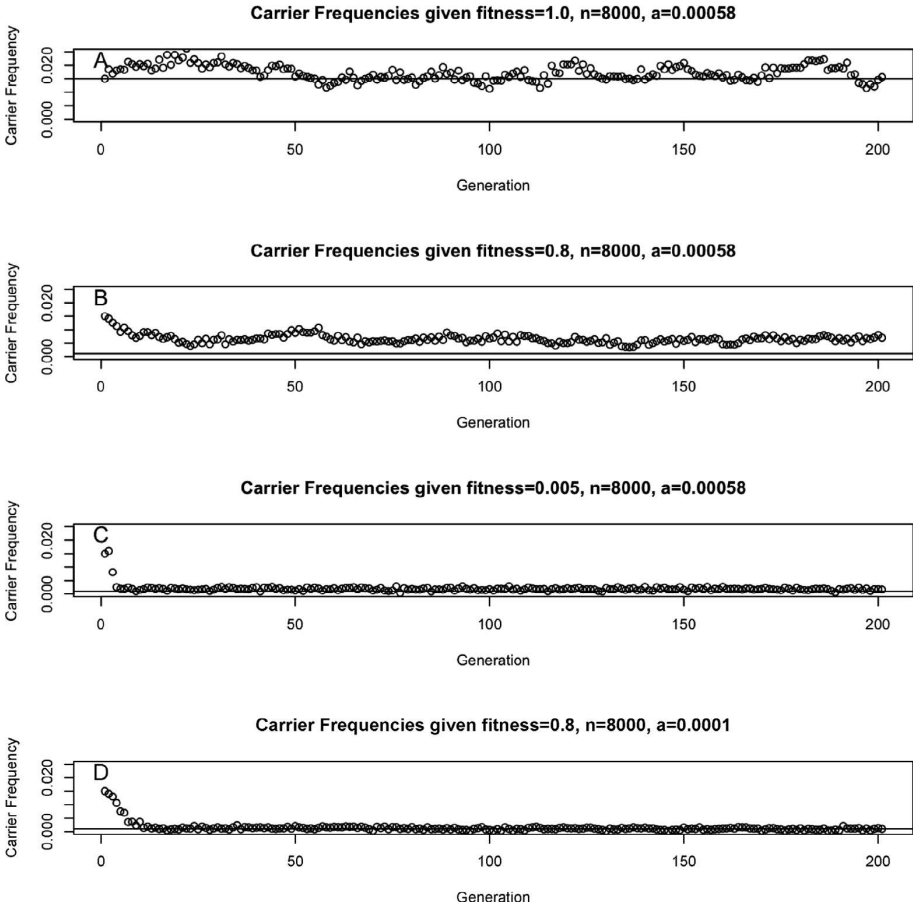

Fig. 6. Modeling how an increase in mean maternal age impacts carrier frequency under various assumptions about mutation rates and carrier fitness. We used our model to examine the trajectories of carrier frequencies in a finite population of 8000 individuals, half of them female, under various assumptions about carrier fitness and mutation rates. A population with mutation rate 0.00058 fluctuates around equilibrium frequency of 0.0145 (horizontal line) under the assumption that carriers have perfect fitness of 1.0 (A). When carrier fitness declines to 0.8 (B), as might be plausible for individuals with FXPOI in a population transitioning to delayed reproduction, carrier frequency declines, and fluctuates around ~0.005 by generation 25. This value is five times the 0.001 carrier frequency (horizontal line) we calculate above for Asian populations. To attain this lower equilibrium value through reduced fitness alone would require a biologically implausible carrier fitness of 0.005 (C), under the assumption of the 0.00058 mutation rate we infer for non-Asian populations. A carrier frequency of 0.001 also could be attained with slightly reduced fitness of 0.8 (D) when combined with the reduced mutation rate of 0.0001 inferred above for Asian populations.

model, a reduction of carrier fitness from 1.0 to 0.8 reduced carrier frequencies by only 2.5 fold. Thus, in both finite and infinite populations, a fitness reduction of this magnitude is not on its own sufficient to account for the six-fold carrier-frequency difference we calculate using empirical data from Asian as compared to non-Asian populations (Table 4).

We found that marked reductions in allele frequencies owing to fitness differences could be attained only when carrier fitness was reduced to 0.005 (Fig. 6C) --- a value that is almost certainly too low even for populations with very high mean maternal age, especially given that only a subset of premutation carriers experience FXPOI. We conclude that reduced fitness alone is likely insufficient to explain frequency differences observed for Asian as compared to non-Asian populations.

A further simulation revealed that lower carrier frequencies observed for some Asian as compared to non-Asian populations likely arise through a combination of reduced carrier fitness and lower mutation rates: assuming carrier fitness of 0.8 and a normal-to-premutation rate of 0.0001, as inferred for Asian populations, carrier frequencies declined within just a few generations to values similar to those observed for Asian populations (Fig. 6D). Thus, fitness differences may produce these frequency differences in concert with mutation-rate differences, but are unlikely to do so on their own.

CONCLUDING REMARKS

Our analysis highlights two factors likely critical in determining *FMR1* carrier frequencies and their differences between Asian and non-Asian populations:

(*i*) Global variation in the rate of normal-to-premutation events

We find that variation in the rate of normal-to-premutation events may contribute substantially to contemporary differences in carrier frequencies reported for Asian as compared to non-Asian populations. Under our model, even minor variation in this mutation rate yields markedly different carrier frequencies. We find that, by contrast, any founder-allele effects that existed when these two groups were established many millennia ago would have disappeared within just a few hundred years.

What might be the origins of variation in *FMR1* normal-to-premutation rates around the world? Perhaps differences in these rates existed at the founding of various populations, as proposed previously (Richards et al., 1992; Chakravarti, 1992; Jacobs et al., 1993). Alternatively, different rates may have arisen after the establishment of these populations, either through mutation or environmental differences. Direct estimates of normal-to-premutation rates at *FMR1* are now available for population-based data collected in the U.S. (Cronister et al., 2008; Nolin et al., 2011; Genereux and Laird, 2013), and fall within the range inferred under our model. It will be important to obtain analogous estimates of *FMR1* mutation rates for Asian populations. Precise estimation of these rates, however, will require appreciably larger sample sizes than those used for U.S. populations if the true mutation rates in Asian populations are as low as those we infer above.

Molecular data on the substructure of the CGG-repeat region provide insight into the origins of this inferred variation in expansion probabilities. The presence of AGG interruptions within the CGG-repeat region has been found to increase allelic stability (Kunst and Warren, 1994; Eicher et al., 1995). New findings from Nolin et al. (2011) demonstrate that, even for alleles with repeat counts in the low 30 s, there is a tendency for stability to decrease with reductions in AGG-repeat counts. Haplotype effects from flanking loci (Curlis et al., 2005), perhaps indicating trans-acting phenomena, may also contribute to disparate mutation rates, as could environmental factors yet to be identified.

Are expansion rates at other triplet-repeat loci also elevated for non-Asian as compared to Asian populations? Warby et al. (2011) suggested that the Huntington Disease locus, *HTT,* like *FMR1,* has a higher rate of expansion of CAG repeats in non-Asian than in Asian populations. By contrast, the dentatorubropallidoluysian atrophy locus, *DRPLA*, has a lower rate of expansion for non-Asian as compared to Asian populations (Deka et al., 1995; Yanagisawa et al., 1996). Thus neither Asian nor non-Asian populations have systematically higher rates of triplet-repeat expansion, suggesting that these expansion probabilities are determined at the level of individual loci.

Could population-specific mutation rates at the *FMR1, HTT,* and *DRPLA* loci derive in part from environmental factors in those populations? It will be informative to assess mutation rates in people of Asian ancestry living in non-Asian countries, and *vice versa*, to investigate whether the mutation rates for specific haplotypes vary with environment.

(*ii*) Reduced fertility of female premutation carriers and mean maternal age

Vogel et al. noted in 1990 that future demographic changes could alter the fitness of *FMR1* premutation carriers, potentially leading genotype frequencies to diverge from existing equilibria. Ongoing increases in mean maternal age represent one such demographic change. FXPOI limits fertility in some female *FMR1* premutation carriers, potentially leading to reductions in mean family size. The fitness of carriers is predicted to be especially low in populations that, like Japan, have high mean maternal age. Analysis under our model revealed that a moderate decline in the fitness of female premutation carriers would indeed yield a decline in carrier frequencies. Only a few more generations would then be required to reach a new, lower equilibrium. Because mean maternal age is increasing in many populations around the world, it is possible that the frequencies of expanded *FMR1* alleles are currently or will soon be out of equilibrium in many populations. Increases in mean maternal age thus may augment the effects of reproductive decision making (see, e.g., Berkenstadt et al., 2007) in reducing the frequency of premutation and full-mutation alleles at *FMR1*.

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