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Predisposition to the Fragile X Syndrome in Jews of Tunisian Descent Is Due to the Absence of AGG Interruptions on a Rare Mediterranean Haplotype

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Summary

We have studied the ethnic distribution of the fragile X syndrome in Israel and have found that 36/136 (26.5%) of apparently unrelated pedigrees were of Tunisian Jewish descent. The Tunisian Jews, however, constitute only 2%–3% of the general Israeli population, identifying the first ethnic group significantly ($P < .001$) predisposed to the development of this disease. Associated with this increase in disease prevalence, we have found an unusually high incidence of FMR1 CGG repeats devoid of AGG interruptions among the normal Tunisian Jewish population (30/150, or 20.0%). Furthermore, the proportion of these alleles beyond the FMR1 CGG repeat instability threshold (>35 repeats) (8/150, or 5.3%) was significantly greater ($P < .04$) than that proportion found among non-Tunisian Jewish controls in Israel (1/136). Haplotype analysis has indicated that these large uninterrupted CGG repeat alleles are present on a previously unreported (DXS548–FRAXAC1–FRAXAC2) haplotype that accounts for all observed cases of disease among Tunisian Jewish X chromosomes. The high prevalence of disease among Tunisian Jews, we suggest, is due to a founder effect of this rare haplotype, which is completely devoid of AGG interruptions in the Jewish population of Tunisia.

Introduction

Worldwide cytogenetic surveys of the fragile X syndrome have found the disease among diverse ethnic groups representing Caucasian, Amerindian, African, and Asian populations (Rhoads 1984; Venter et al. 1984; Bundey et al. 1985; Arinami et al. 1986; Jacobs et al. 1986; Li et al. 1988; Aoi et al. 1989). These data have led to the conclusion that there is no ethnic predilection to the development of the disease (Richards et al. 1994). The high incidence of the disease (Turner et al. 1980; Webb et al. 1986*a*, 1986*b*) and the absence of new mutations (progression from a normal allele [<55 repeats] to the disease state [>200 repeats] in a single human pedigree) suggested that predisposition to expansion may be carried silently through a human lineage for many generations before undergoing the transition to the hyperexpanded state (Morton and Macpherson 1992). Since instability is a hallmark feature of premutation alleles (chromosomes that progress rapidly to disease state), several groups have investigated the molecular basis of unstable alleles in the human population by determining the AGG substructure of various FMR1 CGG repeat alleles (Eichler et al. 1994; Hirst et al. 1994; Kunst and Warren 1994; Snow et al. 1994; Zhong et al. 1995). The majority of premutations were shown to have lost one or both of their AGG interruptions, in contrast to normal stable alleles, which typically possess two AGG interspersions, occurring with a periodicity of once every 9–10 CGG repeat units (Eichler et al. 1994; Hirst et al. 1994; Kunst and Warren 1994; Snow et al. 1994). Furthermore, a comparison of stable and unstable alleles of similar size in the general population (<55 total repeats) revealed that all unstable alleles had lost one or both of their AGG interruptions (Eichler et al. 1994). Significant intergenerational instability was shown to initiate at a threshold of 35 pure repeats, and hyperexpansion was suggested to occur at ~70 pure repeats, requiring a female germ-line transmission to progress to the disease state (Eichler et al. 1994). These data suggested that the loss of AGG interruptions was an important event in predisposing normal alleles to the development of the fragile X syndrome.

We have recently investigated the prevalence of the fragile X syndrome in the Israeli population. Because of

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its recent founding, the ethnicity of the Israeli nation is diverse and complex, consisting of a composite of distinct Jewish and Arab peoples of various origins. In general, the population may be subdivided into three groups: Ashkenazic Jews (Jews of northern European origin); Sephardic Jews (Jews whose origins lie within the Mediterranean basin); and Arabs (Zlotogora and Chemke 1995). We have studied the prevalence of the fragile X syndrome within this ethnic context and have found an unusually high proportion of disease among Tunisian Jews (a subgroup of Sephardic Jews). Henceforth, the term "Tunisian Jew" will be used to refer to a distinct ethnic group, and the term "Sephardic" will be restricted to Jews of Mediterranean origin, excluding Tunisian Jews. Although the Tunisian Jews represent only 2%–3% of the general Israeli population, 36 of 136 unrelated fragile X pedigrees were of Tunisian Jewish ethnicity. This represents a significant ($P < 0.001$) 10-fold enrichment of disease on this ethnic background, indicating an unprecedented predilection to the fragile X syndrome. Since the substructure of the FMR1 CGG repeat has been cited as an important factor in predisposing alleles to instability and disease (Eichler et al. 1994; Hirst et al. 1994; Kunst and Warren 1994; Snow et al. 1994; Zhong et al. 1995), we determined the AGG interspersion pattern of 286 FMR1 CGG repeat alleles from the various Israeli ethnic groups. Our analysis reveals an unusually large number of lengthy (>35 repeats) FMR1 CGG repeat alleles completely devoid of AGG interruptions among the Tunisians. Haplotype analysis confirms that these alleles occur on a rare chromosomal haplotype common to the fragile X syndrome in this population. Our data suggest that founder effects involving this unusual haplotype may explain both the high incidence of FMR1 CGG repeat alleles devoid of interruptions and the prevalence of disease among Tunisian Jews.

Material and Methods

DNA Samples

DNA from fragile X patients was obtained from 136 unrelated pedigrees, diagnosed at six medical centers across Israel, namely, Bnai-Zion Medical Center, Haifa; Kaplan Medical Center, Rechovot; Haddasa Ein Karem Medical Center, Jerusalem; Shiba Medical Center, Tel-Aviv; Soroka Medical Center, Beer Sheba; and Sheba Medical Center, Tel-Aviv. Use of DNA material obtained from human subjects was approved by the appropriate Israeli medical review boards. Standard DNA extraction protocols were used in the collection of DNA from blood samples. Southern blot analysis was used in the detection of fragile X mutations, by use of previously described protocols (Fu et al. 1991). The remaining 239 DNA samples were obtained from unaffected individuals representing the four broadly defined ethnic groups of Israel, as follows: Ashkenazim (44 males), Sephardim (47 males), Arabs (45 males), and Tunisian Jews (42 males and 61 females; total = 164 alleles; 10 female alleles were excluded, because of an inability to unambiguously resolve AGG interspersion pattern). Among the three premutation alleles and one fragile X allele detected among the unaffected Tunisian Jewish control group, an interspersion pattern could be determined for only one premutation allele (67 pure repeats; table 1). The countries of ethnic origin for the Sephardic samples were determined (fig. 1): Algeria (1 sample), Ethiopia (3), Iran (1), Iraq (9), Libya (3); Morocco (27), Turkey (1), Yemen (1), and 1 Sephardic sample of undetermined origin. Among the Tunisian samples, an effort was made to determine the communities from which the families originated. Individuals were selected in whom there was no familial history of mental retardation. Samples were grouped as follows: Isle of Djerba (19 males and 29 females); not from the Isle of Djerba, including the cities of Tatwin, Nabeul, Sus, and Tunis (22 males and 31 females); and Tunisian Jewish samples of unknown origin (1 male and 1 female) (see fig. 2).

AGG Interspersion Analysis

AGG interspersion patterns for 286 FMR1 CGG repeat alleles were determined by *MnlI* restriction-enzyme digestion and CGG repeat oligonucleotide hybridization as described by Eichler et al. (1994). In some cases, determination of AGG interspersion patterns among Tunisian Jewish females was done by performing *MnlI* restriction analysis on the father's DNA and deducing the maternal allele's configuration from the total length of the second FMR1 CGG repeat allele and the remaining hybridizing *MnlI* fragments. When paternal DNA samples were not available, total repeat length of the FMR1 CGG repeat allele, combined with complete and partial *MnlI* restriction analysis, was used to deduce the AGG configuration. The interspersion pattern of some female alleles could not be unambiguously determined. FMR1 CGG repeat interspersion patterns are summarized as follows: a plus sign (+) designates the position of an AGG interruption and the number refers to the triplet length of uninterrupted CGG repeats. For example, an allele configuration designated 9+9+9 contains two AGG interruptions occurring with a periodicity of once every nine CGG repeats.

Haplotype Analysis

Three polymorphic microsatellite markers, DXS548, FRAXAC1, and FRAXAC2, which span 150 kb of the FMR1 locus, were used to reconstruct the chromosomal haplotype of 152 unaffected and 25 fragile X chromosomes. The genotypes for DXS548, FRAXAC1, and FRAXAC2 were determined with previously described

Table 1

AGG Interspersion Patterns of the FMR1 CGG Repeat Allele among Different Israeli Groups

CGG No.	AGG Pattern	TJ	SJ	AJ	Arabs	Total	CGG No.	AGG Pattern	TJ	SJ	AJ	Arabs	Total
17	17	[1]				1	31	9+21				1	1
18	18	[1]				1	32	12+19			1		1
19	10+9	3	2	3	2	10	32	9+12+9	2				2
21	10+10	1				1	33	10+12+9				1	1
21	21				[1]	1	33	13+9+9				1	1
22	12+9			1	1	2	33	9+23		2		1	3
23	23	[1]			[1]	2	35	9+15+9				1	1
23	13+9	3	3	1	4	11	35	9+25		1			1
24	24	[3]				3	36	10+9+15				1	1
24	14+9			1	1	2	36	9+26	1	1			2
25	25	[8]				8	36	9+9+6+9				1	1
25	15+9	1				1	37	9+9+6+10				1	1
26	26	[2]				2	37	37	[1]				1
27	27	[2]				2	38	38		[1]			1
27	7+9+9		1			1	39	19+9+9		1			1
27	9+9+7	1				1	39	9+9+19			1		1
28	9+18			1		1	39	9+9+9+9	3	2	1		6
28	9+8+9	2				2	41	10+9+10+9				1	1
29	29	[1]	[1]			2	42	9+8+23	1				1
29	10+8+9	1				1	42	9+9+22	1	1			2
29	19+9	2				2	43	43	[1]				1
29	9+19	1		2	1	5	43	9+9+23	3	1		1	5
29	9+9+9	43	6	3	11	63	44	10+9+23	1				1
30	10+19	1			1	2	44	10+10+22			1		1
30	10+9+9	34	18	25	8	85	45	46	[1]				1
30	20+9	3				3	45	9+9+25				1	1
30	9+10+9	9		2		11	47	47	[3]				3
30	9+20	1			1	1	48	9+9+27		1			1
31	31	[3]				3	51	51	[1]				1
31	10+9+10		1	1		2	51	9+41		1			1
31	11+9+9		1			1	67	67	[1]				1
31	21+9	1			1	2	Total		150	47	44	45	286
31	9+11+9	1	2		1	4							

NOTE.—The different ethnic groups are arranged in columns, where TJ = Tunisian Jews, SJ = Sephardic Jews, and AJ = Ashkenazic Jews. Interspersion patterns are indicated in the row headings, arranged in ascending order of total repeat length. The number of alleles for each interspersion pattern within each ethnic group is summarized. Cells representing FMR1 CGG repeat alleles devoid of interruptions are boxed. Uninterrupted alleles beyond the instability threshold (>35 repeats) are underlined.

PCR conditions and fluorescent primers with the aid of an ABI 373 automated sequencer (Murray et al. 1996). For each polymorphic marker, alleles were designated with numbers in descending order of repeat length. Because of the complex nature of the FRAXAC2 marker, which consists of two dinucleotide polymorphic repeat tracts and a polymorphic poly T tract (Zhong et al. 1993), care was taken to distinguish between FRAXAC2 alleles, which differ by a single base pair (bp). These are indicated in this study by a plus sign. For example, a FRAXAC2 genotype "4+" is intermediate in size between genotype 3 and 4 differing by a single bp (fig. 3). Similarly, the novel DXS548 haplotype (7+) found in this study refers to an unusual allele length of 193 bp for this marker. Composite haplotypes using all three markers are considered in this analysis and are configured on the basis of the order of each marker, centromere to distal on the long arm of the X chromosome (i.e., centromere–DXS548–FRAXAC1–FRAXAC2–telomere).

Population Statistics

The ethnic distribution of the Israeli population was calculated from data provided by the National Statistical Registry of Israel (1993 census).

Results

Figure 1 compares the prevalence of the fragile X syndrome among four ethnic groups (Tunisian Jews, Sephardic Jews, Ashkenazic Jews, and Arabs) and their representation in the Israeli population. The prevalence

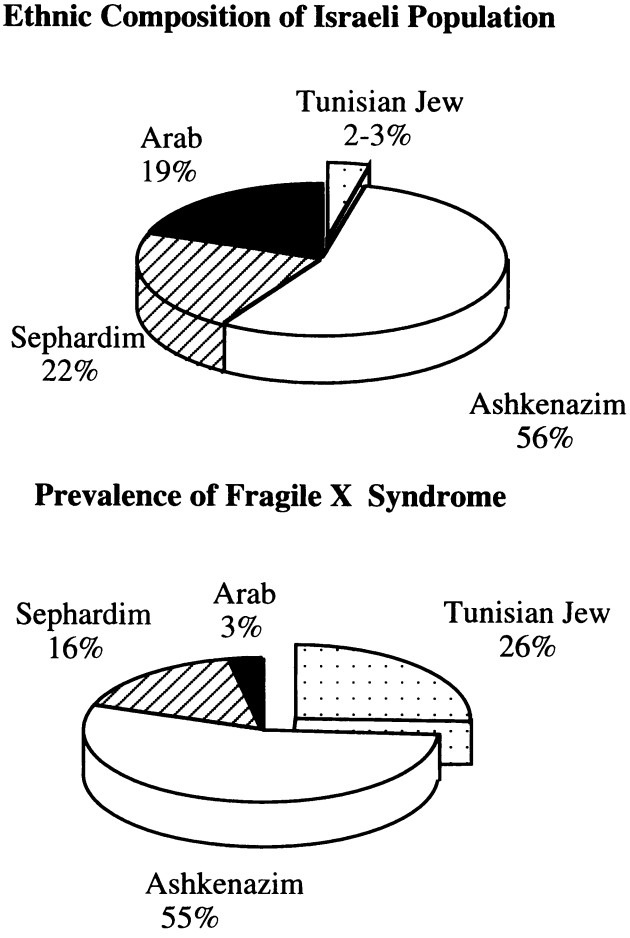

Figure 1 Ethnic distribution of the fragile X syndrome among different Israeli ethnic groups. A pie-chart comparison is made between the prevalence of disease among different ethnic groups of Israel (based on 136 unrelated fragile X pedigrees) and the proportion of the population that each ethnic group constitutes. The fragile X mutation occurs ~10 times more frequently among Tunisian Jews than would be expected on the basis of their representation in Israel. The ethnic distribution of the Israeli population was calculated from data obtained from the National Statistical Registry of Israel (1993 census).

of disease for each ethnic group was determined from 136 unrelated fragile X pedigrees. A highly significant (P <0.001) enrichment of the fragile X syndrome is observed among the Tunisian Jews (36/136, or 26.4%, fragile X pedigrees). The prevalence of the fragile X syndrome among the Sephardic and Ashkenazic population was similar to their representation in the general population. In contrast, fewer cases of fragile X syndrome have been observed among Arabs than would be expected on the basis of their representation in Israel (fig. 1). We surveyed 153 Tunisian FMR1 CGG repeat alleles by PCR analysis from unrelated individuals with no family history of mental retardation. In this sample, three premutation-sized alleles (3/154, or 2.0%) were identified with repeat lengths 67, 81, and 103. One unaffected Tunisian female who carried a full mutation allele was identified by Southern blot analysis (1/154, or 0.6%).

The AGG substructure of the FMR1 CGG repeat for 286 unrelated unaffected chromosomes is summarized in table 1 (150 Tunisian Jews; 47 Sephardic Jews; 44 Ashkenazic Jews; and 45 Arab alleles). Figure 2 indicates the geographic origin of samples constituting the Sephardic Jewish population. Tunisian Jewish samples were obtained from Israeli immigrants who traced their ancestry to the Isle of Djerba and the coastal cities of Tunis, Sus, Nabeul, and Tatwin (see Material and Methods and fig. 2). The proportion of FMR1 CGG repeat alleles devoid of AGG interruptions (30/150, or 20%) is significantly greater (P < .0001; one-tailed Fisher's exact test) among the Tunisian Jews than among the other ethnic groups in Israel (4/136, or 2.9%; table 2). Eight of 150 of the Tunisian chromosomes contained uninterrupted FMR1 CGG repeat alleles with tract lengths >35 pure repeats. Once again, this proportion of putative unstable alleles is significantly greater (P < .04) than non-Tunisian controls (1 of 136 chromosomes). Comparisons of AGG substructure in North American and Wessex populations (Eichler et al. 1995, 1996) (n = 409 chromosomes; table 2) confirm that the proportion of long (>35) uninterrupted CGG repeat alleles among the Tunisian Jews is highly significant (P < .0001; one-tailed Fisher's exact test). Wherever possible, the communities from which these individuals emigrated were determined. Half of these alleles (4/8) cluster among Israeli immigrants who can trace their ancestry to the Isle of Djerba (fig. 2). The remaining four alleles originated from the coastal cities of Tunisia (see Material and Methods).

By use of polymorphic markers DXS548, FRAXAC1, and FRAXAC2, which span 150 kb of the FMR1 locus, the haplotypes of 24 fragile X males and 169 unaffected Israeli control individuals were determined (table 3 and fig. 3). Tunisian Jewish fragile X males occur exclusively (10/10) on a single haplotype (7+-4-6+). Seven of these Tunisian fragile X patients traced their ancestry to the Isle of Djerba (table 3). Ashkenazic fragile X mutations, in contrast, occur primarily on the 2-1-3 or 7-3-4+ haplotypes (table 3). The 7+-4-6+ haplotype was not represented among unaffected or fragile X Ashkenazic chromosomes.

We compared the haplotype and AGG interspersion pattern among 40 unrelated and unaffected Tunisian chromosomes (table 4). FMR1 CGG repeat alleles with long (>35 repeats) uninterrupted tracts of pure repeats occurred exclusively on the 7+-4-6+ haplotype (37, 43, and 46 repeats for samples T64, T102, and T15, respectively). In addition, one premutation allele (T58, 103 repeats; AGG interspersion pattern undetermined) that belonged to the 7+-4-6+ haplotype was detected in the

Figure 2 Origin of Sephardic Jewish alleles. The countries of origin for the Israeli Sephardic population surveyed in this study are stippled in the geographic map of the Mediterranean basin. The Tunisian Isle of Djerba and other communities of ancestral origin are indicated in the expanded map of Tunisia.

unaffected Tunisian control group (1/41). It is interesting that three alleles with two AGG interruptions (9+9+9 for allele T11, T51, and T91b; table 4) were observed on this same genetic background. The interspersion pattern of seven Arabic and Sephardic chromosomes of the 7+-4-6+ haplotype revealed FMR1 CGG repeat alleles with one or two AGG interruptions and an overall length of less than 36 repeats (data not shown). The remaining 0-AGG alleles within the normal Tunisian Jewish population occur on a different haplotype 3-3-4. In contrast to the 7+-4-6+ haplotype, all of these uninterrupted alleles are relatively short (<26 repeats; table 4). In addition, this haplotype is not associated with the fragile X syndrome (table 3).

Discussion

We report the first strong ethnic predilection to the development of the fragile X syndrome among a community of Jews tracing their origin from Tunisia. Although Tunisian Jews represent only 2%–3% of the total population of Israel (see Material and Methods), they constitute ~26% of all fragile X cases observed in Israeli clinics. Because of cultural and religious sentiments, the Tunisian Jews are less likely to seek medical care in the diagnosis and treatment of genetic disease. Therefore, it is possible that clinical ascertainment bias favors an underrepresentation of the actual prevalence of the fragile X syndrome among this population. The dearth of the fragile X syndrome among Arabs is likely a reflection of such an ascertainment bias (table 1). The highly significant ($P < .0001$) enrichment of disease among the Tunisian Jews suggests that the fragile X syndrome may be particularly prevalent among these communities. Among the unaffected Tunisian control group, we have found an unusually high incidence of premutation (3/154) and full-mutation (1/154) FMR1

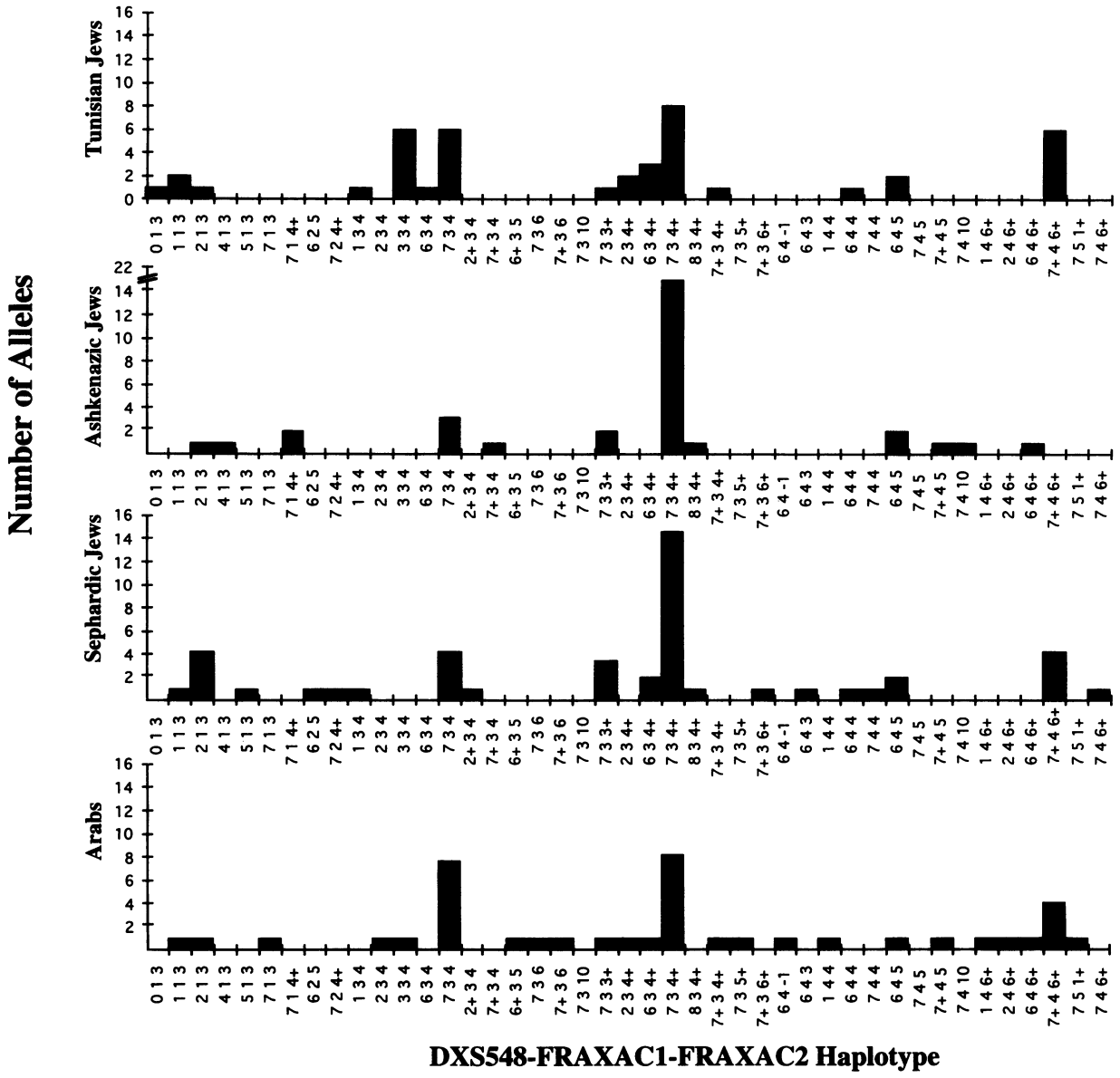

Figure 3 DXS548-FRAXAC1-FRAXAC2 haplotype distribution among Israeli ethnic groups. Haplotypes were determined for 169 unaffected and unrelated Israelis (39 Ashkenazic Jews, 42 Tunisian Jews, 46 Sephardic Jews, and 42 Arabs). Histograms indicate the number of alleles (*vertical axis*) for each DXS548-FRAXAC1-FRAXAC2 haplotype (*horizontal axis*). Haplotypes are grouped in ascending order on the basis of the FRAXAC1 marker (for nomenclature, see Material and Methods).

CGG repeat alleles. These data support a nearly 10-fold increase in the incidence of the fragile X syndrome (Rousseau et al. 1995), with as many as 1/200 births being possibly afflicted with this disease. This defines the first high-risk ethnic group predisposed to the development of the fragile X syndrome. Clinical observation and larger population studies within Tunisia will be necessary to demonstrate more directly this incidence of disease within this ethnic group.

To examine the molecular basis of this disease, AGG interspersion analysis and haplotype studies were performed at the FMR1 CGG repeat locus. We discovered a highly significant ($P < .0001$) proportion (30/150, or 20.0%) of Tunisian Jewish alleles completely devoid of interruptions (tables 1 and 2). Eight of these alleles were beyond the instability threshold of pure repeats (>35), documented in a study of intergenerational transmissions within unaffected normal human pedigrees (Eichler et al. 1994). Haplotype analysis confirms that these long, pure alleles occur on a unique haplotype (7+-4-6+) exclulsively found in association with the fragile X syndrome among the Tunisian Jews (10/10

Table 2

Incidence of FMR1 CGG Repeat Alleles Devoid of Interruptions

Ethnic Group	Proportion of 0-AGG Alleles	Proportion of 0-AGG Alleles >35 Repeats
Tunisian Jew	30/150 (.200)	8/150 (.053)
Non-Tunisian	4/136 (.029)	1/136 (.007)
Ashkenazim	0/44 (0)	0/44 (0)
Sephardim	2/47 (.043)	1/47 (.021)
Arab	2/45 (.044)	0/45 (0)
American/Wessex[a]	9/409 (.022)	0/409 (0)

NOTE.—The table summarizes the proportion (frequency is shown in parentheses) of FMR1 CGG repeat alleles without interruptions for each ethnic group. A separate column is indicated for those alleles that possess >35 uninterrupted CGG repeats. The table compares Tunisian Jews and non-Tunisian ethnic groups represented by the Ashkenazim, Sephardim, and Arabs of Israel (See Material and Methods).

[a] This second control group was taken from a study of AGG interspersion patterns among unaffected individuals (n = 409) from Wessex, England, and Houston, Texas (Eichler et al. 1995, 1996).

pedigrees) (tables 3 and 4). These data suggest that such alleles are highly unstable and likely represent evolving premutations among Tunisian Jews. Indeed, a single premutant allele (71 repeats) was identified in the unaffected control group belonging to the 7+-4-6+ haplotype. Since the loss of AGG interruptions has been speculated to be an important predisposing factor to instability and disease (Eichler et al. 1994; Hirst et al. 1994; Kunst and Warren 1994; Snow et al. 1994), the unusually high frequency of these uninterrupted CGG repeats in conjunction with haplotype data provides a likely molecular explanation for the prevalence of the fragile X syndrome among the Tunisian Jews.

Haplotype analysis indicates that all 0-AGG FMR1 CGG repeat alleles do not have a common genetic background. Two distinct haplotypes emerge in this study that are enriched for FMR1 CGG repeat alleles devoid of interruptions. Tunisian FMR1 CGG repeat alleles without interruption and <26 repeats in length occur on haplotype 3-3-4 (table 4) and are not found in association with the fragile X syndrome (table 3). The 3-3-4 haplotype occurs frequently among Tunisian X chromosomes but is virtually absent among other ethnic groups (fig. 3) (Macpherson et al. 1994; Murray et al. 1996). Tunisian FMR1 CGG repeat alleles devoid of interruptions and >35 repeats in length (table 4) occur exclusively on haplotype 7+-4-6+, which accounts for all Tunisian Jewish disease chromosomes (table 3). These data indicate that the long uninterrupted CGG repeat alleles did not simply progress from the shorter pure CGG repeats within this population. The observation of two Tunisian FMR1 CGG repeat alleles on the 7+-4-6+ haplotype with two AGG interspersions might suggest that long and uninterrupted CGG repeats may have been generated by the loss of these two interruptions (table 1). Since the loss of interruptions has been predicted to occur in a fashion that preserves the overall length of the repeat (Eichler et al. 1995, 1996), an ancestral allele of ~30 pure CGG repeats could have been generated in this manner. Alternatively, a single founder allele devoid of interruptions followed by genetic drift may account for the prevalence of these unstable alleles in this population. If the allele were carried by the original settlers of the Tunisian Jewish community 2,500 years ago, it would suggest that predisposed alleles may "survive" >100 generations before reaching a hyperexpanded state associated with disease and genetic lethality.

All Tunisian fragile X mutations (table 3) occur on a single chromosomal haplotype 7+-4-6+ (table 3). In contrast, Ashkenazic mutations occur primarily on two different DXS548-FRAXAC1-FRAXAC2 haplotypes

Table 3

DXS548-FRAXAC1-FRAXAC2 Haplotype of Israeli Fragile X Chromosomes

Fragile X Patient	DXS548 Allele	FRAXAC1 Allele	FRAXAC2 Allele	Ethnic Origin of Patient
NK17	7+	4	6+	Tunisian
040 I.C.	7+	4	6+	Tunisian/mainland
326 H.U.	7+	4	6+	Tunisian/Djerba
366 A.U.	7+	4	6+	Tunisian/Djerba
374 A.H.	7+	4	6+	Tunisian/Djerba
310 N.C.	7+	4	6+	Tunisian/Djerba
T8	7+	4	6+	Tunisian/Djerba
T10	7+	4	6+	Tunisian/Djerba
T36	7+	4	6+	Tunisian/Djerba
134	7+	4	6+	Tunisian
NK101	7	3	3+	Sephardic/Egypt
NR11	7+	4	6+	Sephardic/Bulgarian
NK31	7+	4	6+	Sephardic/Bulgarian
3018 S.R.	2	1	3	Ashkenazic/Poland
320 S.G.	2	1	3	Ashkenazic/Poland
3008 M.G.	2	1	3	Ashkenazic
007 E.V.	2	1	3	Ashkenazic
3276	2	1	3	Ashkenazic
1341 H.M.	7	1	3	Ashkenazic
3135 R.S.	7	3	4+	Ashkenazic
NK42	7	3	4+	Ashkenazic
3037	7	3	4+	Ashkenazic
388	6	4	5	Arab
3044	6	4	5	Arab

NOTE.—The haplotype and ethnic origin of 24 Israeli fragile X chromosomes is shown. The composite haplotype pattern is summarized in order of DXS548, FRAXAC1, and FRAXAC2 polymorphic dinucleotide markers by use of a previously described nomenclature (Macpherson et al. 1994). The haplotype 7+-4-6+ accounts for all Tunisian fragile X chromosomes.

Table 4

DXS548-FRAXAC1-FRAXAC2 Haplotype of Tunisian Jewish Unaffected Chromosomes

Sample	AGG Pattern	DXS548 Allele	FRAXAC1 Allele	FRAXAC2 Allele
T15	46	7+	4	6+
T64	37	7+	4	6+
T102	43	7+	4	6+
T51	9+9+9	7+	4	6+
T11	9+9+9	7+	4	6+
T91b	9+9+9	7+	4	6+
T68	9+10+9	6	4	5
3053	9+10+9	6	4	5
394	9+9+23	6	4	4
3086	9+26	6	4	4
T20	10+9+9	7	3	4+
T34	10+9+9	7	3	4+
T35	10+9	7	3	4+
324	10+9+9	7	3	4+
T94	10+9+9	7	3	4+
T79	10+9	7	3	4+
T91	10+9+9	7	3	4+
342	9+9+9+9	7	3	4+
T32	10+9+9	7	3	4+
T80	10+9+9	6	3	4+
T104	10+9+9	6	3	4+
T81	10+9+9	6	3	4+
T55	10+9+9	2	3	4+
T73	10+9+9	2	3	4+
1347	21+9	7	3	3+
T54	9+9+9	7	3	4
T62	9+8+9	7	3	4
L43	9+9+9	7	3	4
T84	9+9+9	7	3	4
T53	13+9	7	3	4
T56	25	3	3	4
T69	24	3	3	4
334	25	3	3	4
T92	25	3	3	4
T23	24	3	3	4
T101	9+9+9	1	3	4
T67	9+8+23	2	1	3
T18	9+9+9	1	1	3
T47	9+9+9	1	1	3
340	9+9+9	0	1	3

NOTE.—The haplotype of 40 unrelated and unaffected Tunisian Jewish male individuals is compared to the interspersion pattern of each FMR1 CGG repeat allele. Note that the 7+-4-6+ haplotype background contains all three long, uninterrupted FMR1 CGG repeat alleles devoid of AGG interspersions. 0-AGG alleles with short (<26) uninterrupted stretches of pure repeats occur exclusively on haplotype 3-3-4. Cells representing FMR1 CGG repeat alleles devoid of interruptions are boxed.

(7-3-4+ and 2-1-3 haplotypes; table 3). Haplotype surveys of fragile X chromosomes among Japanese and European populations (Chakravarti 1992; Richards et al. 1992, 1994; Arinami et al. 1993; Buyle et al. 1993; Hirst et al. 1993; Jacobs et al. 1993; Oudet et al. 1993*a*, 1993*b*; Haataja et al. 1994; Macpherson et al. 1994; Malmgren et al. 1994; Zhong et al. 1994) indicate that the fragile X mutation frequently occurs on chromosomes with the 7-3-4+ and 2-1-3 haplotype. Although fragile X mutations on the 7-3-4+ haplotype are similar to the frequency of this haplotype in the general population, the 2-1-3 haplotype demonstrates significant linkage disequilibrium with the disease, particularly among populations of European descent (Oudet et al. 1993*b*; Macpherson et al. 1994; Malmgren et al. 1994; Richards et al. 1994). Our analysis of fragile X haplotypes among the Israeli population reveals that this association is similarly restricted to Jewish populations of European origin (table 3). In contrast, we have found that the 7+-4-6+ haplotype occurs predominantly among peoples of Mediterranean origin (fig. 2), a haplotype which has never been reported among North American, European, Australian, or Japanese populations (Chakravarti 1992; Richards et al. 1992, 1994; Arinami et al. 1993; Oudet et al. 1993*a*, 1993*b*; Buyle et al. 1993; Jacobs et al. 1993; Hirst et al. 1993; Haataja et al. 1994; Macpherson et al. 1994; Malmgren et al. 1994; Zhong et al. 1994). (Reexamination of 729 haplotypes from the Wessex population has found a small [4/729, or 0.6%] but significant number of chromosomes of the 7+-4-6+ haplotype [J. N. MacPherson, personal communication], suggesting that previous studies may have tended to misclassify this unusual haplotype as 7-4-6+.) Although the 7+-4-6+ haplotype occurs frequently among Arabs and Sephardic and Tunisian Jews (fig. 2), only Tunisian Jews possess long, uninterrupted CGG repeat alleles in association with this rare haplotype. This suggests that a strong founder effect for uninterrupted alleles on the 7+-4-6+ background occurred among the original Jewish settlers of Tunisia.

The Tunisian Jews, in general, may be described as a subgroup of Sephardic Jews (Mourant et al. 1978), who are genetically distinct from the Ashkenazic majority of Israel (Tikochinksi et al. 1991; Lucotte et al. 1993). Traditionally, the term "Sephardic" (or *Sepharad*, from Obadiah, verse 20) was used to refer to descendants of the Sephardim, a large Jewish community which prospered in Spain from the 8th to the 15th centuries (Mourant et al. 1978). During the House of Castille and Aragon's reconquest of Spain from the Moors, this Jewish community was expelled from Spain, finding refuge in North Africa, Greece, Turkey, Iraq, and Iran (see fig. 2), becoming assimilated into local Jewish communities in these regions (Mourant et al. 1978). The descendants of these refugees became known as Sephardic, a term which is now generally applied to all Jews whose ancestry heralds from the Mediterranean basin. Although many of the coastal cities of Tunisia accommodated a large proportion of the early Sephardic refugees, certain regions, such as the Isle of Djerba and the plains of

Tafilalet, because of religious and cultural differences received relatively few Sephardic emigrants from Spain (Mourant et al. 1978). The genetic constitution of these isolated regions, as determined by blood-group typing (Moullec and Abdelmoula 1954; Ranque et al. 1964; Mourant et al. 1978) is distinct from neighboring Sephardic and Arabic populations, suggesting descent from a more ancient Jewish population in these areas (Ranque et al. 1964). We have determined the ethnic origin of the Tunisian Jewish samples used in this survey. It is interesting that a higher proportion of uninterrupted CGG repeat alleles and fragile X mutations (table 3) were found among Tunisian Jews who traced their ancestry to the Isle of Djerba than from other coastal cities of Tunisia. The frequent occurrence of these alleles among descendants from this island indicates that the predisposing haplotype originated with the first Palestinian Jewish settlers of Djerba and Tunisia, who emigrated in the 5th century B.C. under the exile of the Babylonian king Nebuchadnezzar (Mourant et al. 1978). After the burning of the first temple in Palestine, two communities were established in Djerba, Chara el Kabira (descendants of the tribe of Zebulun) and Chara el Zrira (descendants of the Levitical priest Aaron). Intermarriage between these communities and the Sephardic Jewish population on the mainland has been rare. Founder effects and genetic drift within these closed populations of FMR1 CGG repeat alleles predisposed to instability and hyperexpansion may account for the high prevalence of disease among Tunisian Jews and explain its lower prevalence among other Sephardic populations in northern Africa.

Conclusions

Among the Tunisian Jews of Israel, we have identified a strong ($P < .001$) ethnic predilection for the occurrence of the fragile X syndrome. A study of AGG interspersion substructure of the FMR1 CGG repeat reveals an unusually high proportion (20%) of Tunisian Jewish chromosomes ($n = 150$) that are completely devoid of AGG interruptions at the fragile X locus. The largest of such alleles (>35 pure repeats) are found on a characteristic DXS548-FRAXAC1-FRAXAC2 haplotype, which accounts for all fragile X cases studied in this population. These data confirm that the absence of AGG interruptions is a pivotal event in both instability and predisposition to disease. The ethnohistory of the Tunisian Jews support a model of unique founder effect and genetic drift phenomena for the accumulation of predisposed alleles in the population and the high prevalence of the fragile X syndrome among this particular group of Sephardic Jews. Furthermore, the high incidence of premutation (3/154) and fragile X mutations (1/154) in this population may warrant the development of a more comprehensive screening program among Tunisian Jews in Israel.

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