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Strong Founder Effect for the Fragile X Syndrome in Sweden

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Abstract

We analyzed the FRAXAC2 and DXS548 microsatellites in normal and fragile X chromosomes from Sweden and the Czech Republic in order to investigate a possible founder effect for chromosomes carrying a fragile X mutation. We report a much stronger linkage disequilibrium between the marker haplotypes and the disease in Swedish fragile X chromosomes than in Czech and most other previously studied Caucasian populations. Two haplotypes accounted for 64% of Swedish fragile X chromosomes and for only 14% of normal chromosomes. Neither of these two haplotypes was found in Czech chromosomes, but the most common Swedish fragile X haplotype is the same as that reported to be predominant in Finnish fragile X patients. Linkage disequilibrium was observed in the Czech fragile X chromosomes but the haplotypes were more diverse and similar to those observed in other Caucasian populations. The most prevalent Swedish fragile X haplotype was traced back from affected males to common ancestors in the early 18th century. This indicates an apparently silent segregation of fragile X alleles through up to nine generations. The geographical distribution of the two major at-risk haplotypes in Sweden suggests that they were present among early settlers in different parts of the country.

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Key Words

Fragile X syndrome
Microsatellites
Linkage disequilibrium
Founder effect

Introduction

The fragile X syndrome, the most common type of inherited mental retardation, is caused by the expansion of an unstable CGG repeat

sequence located in a 5' exon of the FMR1 gene [1-3]. The CGG repeat varies in length in the normal population with allele sizes ranging from 6 to 54 [4]. Two types of allele can be distinguished in fragile X families: a

premutation (60–200 repeats) present in intellectually normal carriers and a full mutation (>200 repeats) present in fragile X patients [5]. The transition from premutation to full mutation occurs only when transmitted by a carrier female to her offspring, and the probability of the event depends on the size of the premutation [4, 6, 7]. Because observations of direct passage from a normal allele to a premutation are lacking, no mutation rate has been determined for the first premutation event. It has been speculated that premutated fragile X chromosomes may be passed through several generations before a full mutation occurs [8, 9]. Recent investigations using microsatellite markers FRAXAC1 and FRAXAC2 flanking the CGG repeat have shown haplotypes in linkage disequilibrium with fragile X mutations [10, 11]. Three haplotypes were found to be significantly overrepresented in Australian and US populations, accounting for 58% of fragile X chromosomes. Similar findings were reported from a French study where the markers FRAXAC2 and DXS548 showed two haplotypes overrepresented on fragile X chromosomes [12]. This suggests that certain chromosomes may be susceptible to develop the full mutation present in fragile X patients. The initial event(s) leading to a premutation has presumably occurred on a limited number of chromosomes and has remained silent over many generations. This is an unexpected finding in a relatively common X-linked disease that decreases reproductive fitness [13].

We have used the microsatellite markers FRAXAC2 and DXS548 flanking the CGG repeat in Swedish and Czech populations to search for linkage disequilibrium. The FRAXAC2 marker is located only 10 kb distal, and DXS548 150 kb proximal to the CGG repeat [2, 10, 14]. We show that the putative founder effect in Sweden is much stronger than that observed in the Czech Republic and

in other previously studied populations of central European origin [11, 12]. Genealogical information and results of haplotype analysis were combined for a few affected individuals, allowing us to follow the mutation back through several generations of silent carriers. Our combined results indicate that the initial mutation events that create susceptibility to further expansion are rare, in agreement with the hypothesis of a founder effect.

Patients and Methods

Typing of CA repeats FRAXAC2 and DXS548, both highly informative microsatellites [2, 10, 14], was performed on a population of 28 Swedish and 15 Czech fragile X patients. The healthy chromosomes of their mothers or in some cases fathers or unrelated males were used as reference haplotypes. Close relationship between affected individuals was excluded, i.e. up to third or fourth cousin. The diagnosis of fragile X syndrome was made on the basis of clinical criteria, standard cytogenetic procedures and results of DNA analysis with the probe StB12.3 [15]. The polymerase chain reaction (PCR) was performed using oligonucleotide primers for the FRAXAC2 and DXS548 loci after end-labeling of one primer with ^{32}P . Amplification by PCR was carried out simultaneously for the two loci as described by Oudet et al. [16]. The PCR products were diluted 1:25, denatured and separated on a 6% denaturing polyacrylamide gel, with an M13mp18 sequence marker for sizing. Consistency of allele denomination was ensured by typing control DNAs used in previous studies [12, 16]. For calculations of statistical significance we used χ^2 tests with correction for continuity.

Results

We haplotyped 28 Swedish normal and fragile X chromosomes as well as 20 normal and 15 fragile X chromosomes in the Czech Republic.

The apparent sizes of the FRAXAC2 alleles in our control sample extended from 146 to 153 bp and the alleles were separated by

Table 1. Distribution of alleles at FRAXAC2 and DXS548 loci in normal and fragile X chromosomes from different countries

Allele	Normal chromosomes						Fragile X chromosome					
	Swedish (n = 28)		Czech (n = 20)		French ^a	Australian/ American ^b	Swedish (n = 28)		Czech (n = 15)		French ^a	Australian/ American ^b
	%	n	%	n	%	%	%	n	%	n	%	%
<i>FRAXAC2</i>												
146 (A)	7	2	5	1	10	8	7	2	0		7	3-11
147 (B)	7	2*	5	1	6	8	32	9*	20	3	8	11-23
148 (C)	0		0		0.5	5	0		0		1.5	0-12
149 (D)	14	4	20	4	17	18	7	2	33	5	30	8-38
150 (E)	54	15	65	13*	49	52	50	14	27	4*	21	13-31
151 (F)	11	3	5	1	9	8	4	1	20	3	29	21-47
152 (I)	4	1	0		1	0	0		0		0	0
153 (K)	4	1	0		1	0	0		0		0	0
<i>DXS548</i>												
192 (b)	0		5	1	2	-	0		7	1	0	-
194 (c)	79	22*	75	15**	72	-	50	14*	20	3**	39	-
196 (d)	14	4**	5	1	14	-	46	13**	27	4	30	-
202 (g)	0		0		2	-	0		0		1	-
204 (h)	7	2	15	3	9	-	4	1	47	7	27	-

Departure between frequencies within normal chromosomes and those within fragile X chromosomes have been tested with a χ^2 test of independence. Significance levels for comparisons between the fragile X and normal chromosomes are indicated (* $p < 5 \times 10^{-2}$, ** $p < 10^{-2}$). Allele sizes for FRAXAC2 were adjusted in accordance to Zhong et al. [17].

^a From Oudet et al. [16].

^b From Richards et al. [11] and Zhong et al. [17].

one nucleotide as reported recently by Zhong et al. [17]. The most frequent allele in the control populations was 150 bp (E allele). The polymorphism displayed a bimodal distribution (table 1). The apparent sizes of the DXS548 polymorphism extended from 192 to 204 bp in our controls. The alleles were separated by two base pairs with a bimodal distribution.

The distribution of normal alleles for the two markers was similar when comparing the Swedish and Czech populations. There were slight deviations, but the populations were too

small to test whether such differences are significant between these two populations, and also in comparison to previously studied Australian, US and French populations [11, 12, 17]. In contrast, significant differences were observed when comparing the normal alleles and the alleles on fragile X chromosomes within both populations (table 1).

In both cohorts the major haplotypes on fragile X chromosomes were distinct from those on normal chromosomes. In the Swedish population 10 different haplotypes were observed on normal chromosomes and 7 on

Table 2. Distribution of FRAXAC2-DXS548 haplotypes in fragile X and normal chromosomes from different countries

Haplotype	Normal					Fragile X				
	Swedish		Czech		French ^a	Swedish		Czech		French ^a
	%	n	%	n		%	%	n	%	
<i>FRAXAC2-DXS548</i>										
146-194 (Ac)	7	2	5	1	5	7	2	0		3
147-194 (Bc)	4	1*	0		3	29	8*	0		0
147-196 (Bd)	4	1	5	1**	4	4	1	20	3**	9
149-194 (Dc)	14	4	15	3	11	0		0		9
149-196 (Dd)	0		0		2	7	2	7	1	11
149-204 (Dh)	0		5	1**	2	0		27	4**	9
150-192 (Eb)	0		5	1	1	0		7	1	1
150-194 (Ec)	43	12*	55	11*	44	14	4*	20	3*	16
150-196 (Ed)	11	3*	0		5	36	10*	0		1
150-204 (Eh)	0		5	1	4	0		0		3
151-194 (Fc)	4	1	0		5	0		0		12
151-204 (Fh)	7	2	5	1**	1	4	1	20	3**	14
152-194 (Ic)	4	1	0		0	0		0		0
153-194 (Kc)	4	1	0		1	0		0		0

The distribution of Swedish and Czech fragile X chromosomes differed significantly ($p < 10^{-3}$). They differed significantly also when comparing the 147-194 haplotype ($p < 5 \times 10^{-2}$) with the 150-196 haplotype ($p < 10^{-2}$). When comparing normal and fragile X chromosomes within the Czech population a significant difference was detected by pooling the haplotypes 147-196, 149-204 and 151-204 ($p < 10^{-2}$). For statistical methods, see the footnote to table 1. * $p < 5 \times 10^{-2}$, ** $p < 10^{-2}$ when comparing normal and fragile X haplotypes.

^a From Oudet et al. [16].

fragile X chromosomes (table 2). Similarly, the figures in the Czech population were 8 and 6, respectively. The two major haplotypes on control chromosomes, 149-194 (Dc) and 150-194 (Ec) were together observed at a frequency of 57% in Sweden and 70% in the Czech Republic.

In the Swedish cohort, the two most common fragile X haplotypes 147-194 (Bc) and 150-196 (Ed) were significantly more frequent than in controls. None of these were observed in the Czech material where three fragile X haplotypes, 147-196 (Bd), 149-204 (Dh) and 151-204 (Fh) were overrepresented

and together significantly more frequent than in corresponding controls. Similarly, the 150-194 (Ec) haplotype is significantly underrepresented in both fragile X populations. In the Swedish population, the major fragile X haplotype 150-196 (Ed) accounted for 36%, and was found on only 11% of normal chromosomes. The haplotype 147-194 (Bc) was found on 29% of fragile X chromosomes but was observed only once (4%) in normal chromosomes. Taken together, the distribution of haplotypes on fragile X chromosomes in Sweden was significantly different from those observed in the Czech Republic (table 2). The

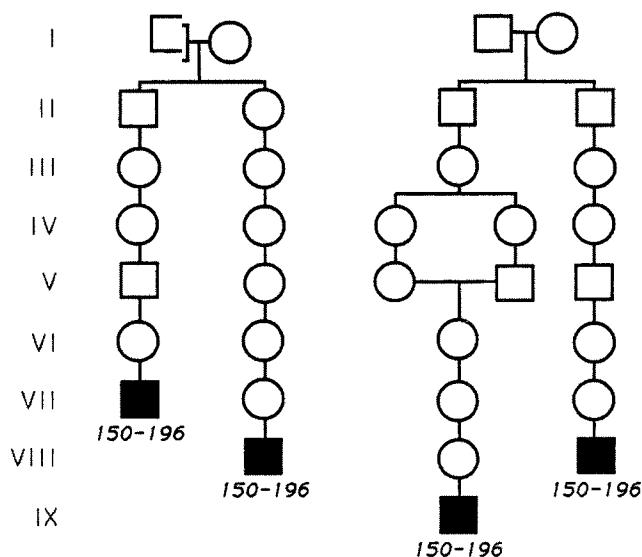


Fig. 1. Two pedigrees with alleles for loci FRAXAC2-DXS548 in individuals segregating for the fragile X syndrome. In each pedigree two affected individuals could be traced back through 7–9 generations to common ancestors living in the early 18th century. The identical haplotype in distantly related affected individuals suggests a silent premutation or a mutant carrier state through the generations.

two major haplotypes which constituted 64% of fragile X chromosomes in Sweden were not found in Czech chromosomes and represent only 1% of French chromosomes [12].

A founder effect was further supported by combining genealogical [8] and molecular analysis of fragile X patients from northern Sweden. Common ancestors of affected individuals with the 150–196 (Ed) haplotype were traced back to the early 18th century (fig. 1). This shows that the mutation has been carried over 8–9 generations of silent carriers.

The geographical distribution of the fragile X haplotype showed variations in Sweden. The predominant haplotype 150–196 (Ed) was seen on 86% of fragile X chromosomes originating from the northern half of Sweden (6 out of 7). The frequency differed significantly from that observed in the southern part where only 19% of fragile X chromosomes (4 out of 21) carried the 150–196 haplotype

($p < 10^{-3}$). The second major haplotype in Sweden, 147–194 (Bc), was found predominantly in the southern half of the country where it is present on 38% of fragile X chromosomes (8 of 21). Southern Sweden also showed a more diverse mixture of haplotypes.

Discussion

The heritable unstable element responsible for the fragile X syndrome was recently demonstrated to be in linkage disequilibrium with flanking microsatellite alleles [11, 12, 16–18]. The existence of a founder effect for the fragile X syndrome was unexpected. Indeed, the fragile X syndrome reduces reproductive fitness of affected males and is found at a high frequency in many populations throughout the world [19]. A high mutation rate would

thus be expected, as observed in other X-linked traits. The difference in frequency of the haplotypes on normal and fragile X chromosomes indicates a limited number of primary mutational events. This putative founder effect was tested in Swedish and Czech populations that share no recent demographic interaction. We have shown that the fragile X mutation in Sweden is associated with a stronger founder effect than that observed in the Czech Republic and previously reported for France [12]. Two major haplotypes account for 64% of Swedish fragile X chromosomes but only 14% of normal chromosomes. The two haplotypes were not found in any Czech patients and have previously been reported on only 1% of a French fragile X population [12]. By contrast, the distribution of haplotypes on fragile X chromosomes in the Czech population is more similar to that observed in France which might reflect a recent demographic interaction of the populations of central Europe, whereas the Scandinavian peninsula has remained more isolated.

Furthermore, we have demonstrated that the most prevalent fragile X haplotype in the Swedish population can be traced to common ancestors 8–9 generations back. This supports the assumption that the premutation can be carried through many generations by silent carriers. However, the initial event that produced the premutated founder chromosome must have occurred much earlier in history [13].

It has been suggested that part of the complexity observed for the haplotypes flanking the fragile X mutation may result from a higher mutation rate of the microsatellite markers compared to biallelic restriction fragment Length polymorphisms. Polymerase slippage has been proposed to account for the heterogeneity of fragile X haplotypes [12]. New mutations at the FRAXAC2 locus have indeed been reported [17] but have not been observed in our material. Furthermore, with a

distance between the two markers of about 160 kb, recombination can occur, although at a very low frequency.

Mutations that alter the length of the CGG repeat at the FMR1 gene are likely to be rare but not unique events. A mutation rate of 2.5×10^{-4} has been suggested [13]. This would generate different risk haplotypes in different populations. Our results comparing fragile X and normal chromosomes between Sweden and the Czech Republic supports this hypothesis. One or a few founders in Sweden for the most prevalent fragile X haplotype 150–196 (Ed) is the most probable explanation, as the haplotype is apparently very rare on fragile X chromosomes in other populations. This haplotype could originally have arisen by single slippage from the 150–194 (Ec) allele which seems to be predominant in other Caucasian fragile X populations. The second most prevalent fragile X haplotype in Sweden, 147–194 (Bc), which has not been seen in other Caucasian populations studied, may have been derived through an ancient recombination event or may represent an independent mutational event at the CGG repeat. Interestingly, the most common haplotype, 150–196 (Ed), in the Swedish fragile X population is even more predominant in Finnish fragile X patients [17; J. Leisti, pers. commun.] indicating a common origin for part of the Swedish and Finnish fragile X patients.

The predominant population of the Scandinavian peninsula is thought to derive from two prehistorical immigrations, about 10,000 and 6,000 years ago. Since then, immigrations have taken place but of less numerical importance. A possible explanation for the haplotype differences within Sweden, with a more homogeneous pattern in the north, is that this region has had a small and stable gene pool with little demographic movement. The second predominant haplotype in the Swedish fragile X population, 147–194 (Bc), was prob-

ably present among early settlers in the southern part of the country. The more diverse haplotype pattern found in the south is probably due to additional founders of more recent arrival in Sweden, and these haplotypes are also found in a higher proportion in other Caucasian fragile X populations.

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