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Fragile X founder effect and distribution of CGG repeats among the mentally retarded population of Andalusia, South Spain

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Abstract

Fragile X syndrome is the most common inherited form of mental retardation. We investigated the prevalence of the Fragile X syndrome in the population with mental retardation of unknown etiology in Andalusia, South Spain. We analyzed 322 unrelated patients (280 males and 42 females), and found a fragile X syndrome frequency of 6.5%. Among the non-fragile X chromosomes, the 29 CGG repeat was the most common allele. At the linked microsatellite DXS548 locus, we found a new allele which we called “allele 10” (17 CA). Similar to other south European populations, allele 2 (25 CA) at the DXS548 locus and the fragile X allele were in linkage disequilibrium supporting the idea of a common founder chromosome predisposing to the CGG expansion.

Key words: mental retardation, fragile X syndrome, CGG repeats, genetic screening

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Introduction

Fragile X syndrome is the most common cause of hereditary mental retardation. It is characterized by mental handicap, facial dysmorphism and expression of a fragile site at Xq27.3 (Martin and Bell, 1943; Lubs, 1969; Escalante and Frota-Pessoa, 1969; Escalante *et al.*, 1971; Sutherland, 1977; Sutherland and Ashford, 1979; Sherman *et al.*, 1985; Chakrabarti and Davies, 1997; Kooy, 2000). In white populations of European origin its estimated prevalence is 1 in 4,000 males (Turner *et al.*, 1996; Morton *et al.*, 1997). The molecular basis of the fragile X syndrome is an expansion of the (CGG)n triplet repeats located within the 5’ UTR region of the FMR-1 gene, resulting in the absence of the encoded protein (FMRP), which is a ribosome-associated RNA-binding protein (Verkerk *et al.*, 1991; Fu *et al.*, 1991; Feng *et al.*, 1997; Jin and Warren, 2000). The presence of large expansions (n > 200) is associated with abnormal methylation of the surrounding DNA and suppression of FMR-1 expression and translation (Piretti *et al.*, 1991; de Vries *et al.*, 1997; Willemsen *et al.*, 1997).

The CGG repeats are polymorphic, their mode of distribution varying according to the population studied (Brown *et al.*, 1996; Chiurazzi *et al.*, 1996b; Tzeng *et al.*, 1999; Chiang *et al.*, 1999; Saha *et al.*, 2001). Similar to several other diseases involving dynamic mutations, there is evidence of a founder effect based on the demonstration of linkage disequilibrium between the fragile X locus and its flanking polymorphic markers (Richards *et al.*, 1992; Buyle *et al.*, 1993; Oudet *et al.*, 1993; Macpherson *et al.*, 1994; Zhong *et al.*, 1994a; Zhong *et al.*, 1994b; Chiurazzi *et al.*, 1996c; Eichler and Nelson, 1996; Syrrou *et al.*, 1996; Jara *et al.*, 1998). The two most frequent DXS548/FRAXAC1 haplotypes in fragile X chromosomes (2-1 and 6-4) were found in non-fragile X chromosomes whose CGG repeat structure would predispose to expansions, leading to a founder effect (Eichler *et al.*, 1996).

In this work, we investigated the prevalence of the Fragile X syndrome in subjects with mental retardation of unknown etiology, in Andalusia, South Spain. We also studied the allele frequencies at the linked DXS548 loci in normal and fragile X chromosomes.

Material and Methods

Subjects

This study included 322 unrelated patients (280 males and 42 females) with mental retardation of unknown

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etiology, referred to us by pediatricians, child neurologists, psychiatrists and clinical geneticists. In a subgroup of 142 male patients the DXS548 locus was genotyped and the FRAXA/DXS548 haplotypes, determined. The FRAXA locus was also analyzed in 30 X chromosomes from the normal population.

DNA analysis

DNA was isolated from peripheral blood samples by the salt precipitation method (Miller *et al.*, 1988). Fragile X syndrome was diagnosed by Southern blotting as described previously (Pintado *et al.*, 1995). PCR amplification of the CGG repeats at the FRAXA locus of non-fragile X chromosomes was achieved using the c and f primers described by Fu *et al.* (1991). The analysis of CA repeats at the flanking DXS548 locus was carried out as previously reported (Hallmayer *et al.*, 1994). The aliquots of the PCR products were loaded on 6% denaturing acrylamide gels. Alleles were sized by running in parallel lambda gt11 α-^{35}S-labelled sequencing plasmid or α-^{32}P-labelled pBR322 MspI-digested fragments. To calculate the exact number of CGG repeats at the FRAXA locus, we used different sequenced alleles as reference, one of them with 29 triplets, kindly provided by Dr. B. Oostra (Erasmus University, Rotterdam).

Statistical methods

The significance of the differences between fragile X and control samples at the DXS548 locus was assessed by means of the chi-square test (SigmaStat™ 1.0. Statistical Software).

Results and Discussion

Among the 322 individuals studied, we found 21 with a fragile X (20 males and one female), corresponding to a 6.5% frequency. In previous studies performed on unselected retarded males, the frequencies of affected subjects ranged from 2.9% to 15% (Turner *et al.*, 1986; Mazurczak *et al.*, 1996; Mornet and Simon-Bouy, 1996; Gonzalez-del Angel *et al.*, 2000; Limprasert *et al.*, 2001).

Various studies have revealed an allele with 30 CGG repeats as the most frequent one at the FRAXA locus in Caucasian populations, and the 29 CGG repeats initially reported has been considered a miscalculation due to differences in C+G content which affect the migration of the PCR products (Brown *et al.*, 1996; Chiurazzi *et al.*, 1996b). In order to avoid this artefact, we determined the exact number of CGG repeats in non-fragile X chromosomes by running different sequenced CGG repeat alleles in parallel.

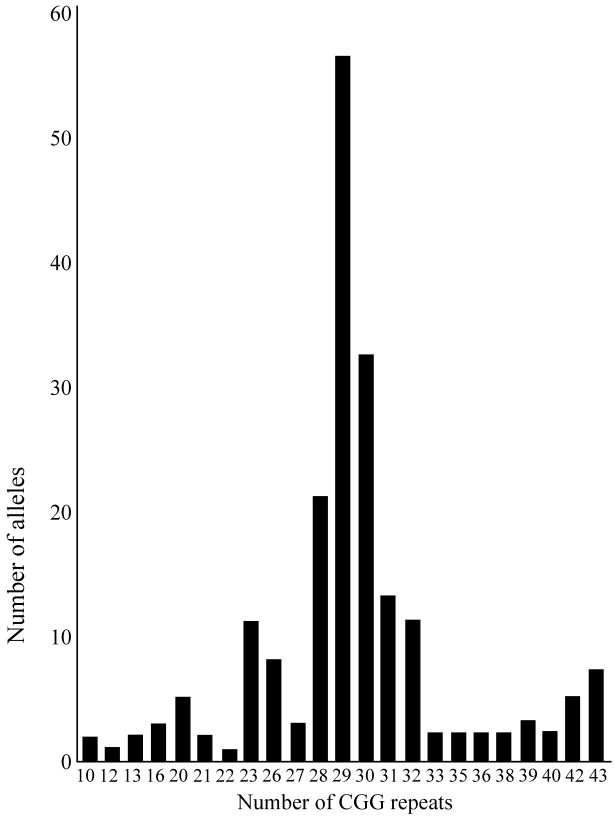

Figure 1 - Distribution of CGG repeats in the non-fragile-X mentally retarded population of Andalusia.

We identified 23 different normal alleles ranging in size from 10 to 43 CGG repeats (Figure 1). Six alleles (with 23, 28, 29, 30, 31 and 32 repeats) accounted for 75% of the total, the 29 triplet allele having been the most frequent one, which is in contrast with the aforementioned studies (Brown *et al.*, 1996; Chiurazzi *et al.*, 1996b). The allele with 29 CGG repeats is also the most frequent allele in the Asian populations (Cheng *et al.*, 1997), although other studies suggest the allele with 28 CGG repeats to be the most common allele in China (Chiang *et al.*, 1999). The 29 CGG allele is also the most frequently reported in India (Saha *et al.*, 2001). Since our study was performed on a population with mental retardation of unknown etiology, it could be argued that this could lead to a bias in the ascertainment of X chromosomes. However, our study of 30 X chromosomes from non-retarded persons, showed similar allele frequencies. Based on these results, we considered that non-fragile-X individuals were representative of the normal population for the FRAXA locus, although a larger number of X chromosomes from our normal population should be studied. Considering the range between 5 and 52 triplets as normal, our sample contained 90% of small alleles (< 35 CGG) and 10% of large alleles (>35 CGG), in agreement with a previous report (Milá *et al.*, 1994).

In order to verify the presence of a founder chromosome, we analyzed the distribution of alleles at DXS548 locus, a polymorphic marker located 150 Kb centromeric from the CGG repeats, that co-segregates, in the majority of the cases, without recombination with the fragile-X locus (Fu *et al.*, 1991; Dreesen *et al.*, 1994). In addition to all the previously described DXS548 alleles, we detected a new allele which we called "allele 10" (17 CA), following the terminology recommended by Macpherson *et al.* (1994) (Figure 2). The frequencies of DXS548 alleles in our sample show a slightly higher genetic diversity than in other populations, which probably reflects Spain's more heterogeneous genetic background, as it has been previously reported for other loci (Bertrantpetit and Cavalli-Sforza, 1991; Cavalli-Sforza and Piazza, 1993; Chillon *et al.*, 1994; Milá *et al.*, 1994; Milá, 1997). In the non-fragile X chromosomes, we observed that the most frequent DXS548 allele was allele 7 (20 CA, 47%), followed by allele 6 (21 AC, 23%) (Table 1). Table 2 shows the DXS548/ (CGG)n-FRAXA haplotypes in the non-fragile X chromosomes, compared to fragile X. Similar to previous work in south European countries and black African populations, allele 2 at the DXS548 locus was present in 52% of non-related fragile-X-positive subjects, whereas it was very uncommon (9%) in non-related fragile-X-negative mentally retarded subjects. Therefore, a statistically significant linkage disequilibrium between the fragile X chromosomes and allele 2 at the DXS548 locus was demonstrated (X^2 = 19.4; p = 0.002; df = 3). No disequilibrium with regard to the normal (CGG)n repeats was detected. These results are consistent with the idea of at least one founder chromosome for the fragile X syndrome in our population, corresponding to one of the original predisposing chromosome in Indo-European populations that could derive from an ancient African founder, as postulated by Chiurazzi *et al.* (1996c).

In summary, we showed that the frequency of fragile X syndrome in our population with mental retardation of unknown etiology was similar to the frequency described in

Figure 2 - PCR-amplified DXS548 alleles: line 4 shows allele 10 (17 CA), not reported previously. pBR322 MspI-digested fragments (M) and gt11 α-^{35}S-labelled sequencing plasmid (GATC) were used for allele sizing.

Table I - Allele distribution at the locus DXS548 in different populations.

DXS548 Alleles (a)	Andalusia Present study	USA Zhong *et al.* (1994[a])	UK MacPherson *et al.* (1994)	Belgium-Holland Buyle *et al.* (1993)	France Oudet *et al.* (1993)	Italy Chiurazzi *et al.* (1996b)	Greece-Cyprus Syrrou *et al.* (1996)	Cameroon Chiurazzi *et al.* (1996c)	China Zhong *et al.* (1994b)
1 (26 CA)	8	3	8	4	2	4	1	-	-
2 (25 CA)	14	15	12	14	15	10	6	1	-
3 (24 CA)	7	7	-	1	1	-	-	9	-
4 (23 CA)	2	-	-	2	1	-	1	3	1
5 (22 CA)	3	2	-	-	-	3	-	4	3
6 (21 CA)	26	18	28	14	23	30	3	12	30
7 (20 CA)	72	139	138	98	117	162	56	44	183
8 (19 CA)	13	6	2	-	3	4	3	1	10
9 (18 CA)	2	-	-	1	-	2	-	-	-
10 (17 CA)	2	-	-	-	-	-	-	-	-
Total	149	190	188	134	162	215	70	74	227

(a) DXS548 alleles named after Macpherson *et al.* (1994).

Table II - DXS548-FRAXA haplotypes in the mentally retarded population of Andalusia.

Fraxa Alleles (CCG)n	DXS548 alleles									
	(17 CA) 10	(18 CA) 9	(19 CA) 8	(20 CA) 7	(21 CA) 6	(22 CA) 5	(23 CA) 4	(24 CA) 3	(25 CA) 2	(26 CA) 1
10									2	
12									1	
16				2						
20	1		1	3	1					
21				1						
23				4	1			1	1	
26				5	1			1		
27				1	1					
28	1			6	2	1			1	
29		1	5	28	9	2	1	2	5	2
30			1	6	3		1		1	1
31		1		7	1			2		2
32			1	1	2			1		
35				1	1					
36				2						
39			1	1					1	
40				1						
42				2	1					
43				1	3				2	
Non fragile - X	2	2	9	72*	26*	3*	2	7	14*	5
Fragile - X				5*	3*	1*			10*	

Non fragile-X chromosomes compared to fragile-X. (χ^2 = 19.4, df =3; p = 0.0002).
DXS548 alleles named after Macpherson *et al.* (1994).

other populations. We found the allele with 29 triplets at the FRAXA locus to be the most frequent one in our geographic area, at least in the mentally retarded non-fragile-X population. We also found a new allele of 17 CA at the DXS548 locus, and showed that the allele frequencies at this locus had a broader distribution than in other populations. As previously reported in south European and black African populations, allele 2 at the DXS548 locus showed linkage disequilibrium with the fragile-X alleles.

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