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.

Keywords: 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; Buyse 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
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 $\alpha$-35S-labelled sequencing plasmid or $\alpha$-32P-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.

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).

![Figure 1 - Distribution of CGG repeats in the non-fragile-X mentally retarded population of Andalusia.](image_url)
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 (Bertranpetit 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- FRA(X) 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 ($\chi^2 = 19.4$; 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. pH322 MspI-digested fragments (M) and g11 α-35S-labelled sequencing plasmid (GATC) were used for allele sizing.](image)

Table 1 - Allele distribution at the locus DXS548 in different populations.

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(b) 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.

Acknowledgments

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References


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

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Non fragile-X chromosomes compared to fragile-X. (χ² = 19.4, df =3; p = 0.0002).

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


Sutherland GR and Ashford PLC (1979) X-linked mental retardation with macro-orchidism and the fragile site at Xq27 or 28. Hum Genet 48:117-120.


