

ORIGINAL ARTICLES

The fragile X syndrome: no evidence for any recent mutations

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Abstract

Fragile X (fra(X)) syndrome, the most common form of familial mental retardation, is caused by heritable unstable DNA composed of CGG repeats. As reproductive fitness of fra(X) patients is severely compromised, a high mutation rate has been proposed to explain the high prevalence. However, we have been unable to show any new mutation for 84 probands referred to us to date. We show here the same fra(X) gene in five fra(X) probands with common ancestors married in 1747. The lack of new fra(X) mutations implies that there must be many more fra(X) gene carriers in the population than previously realised. As it is now possible to detect asymptomatic fra(X) gene carriers by DNA analysis, extended family studies for any new proband are recommended. A family illustrating the importance of fra(X) carriership determination is reported.

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The fra(X) syndrome is the most common form of familial mental retardation. Owing to the severity of the disorder, reproductive fitness is greatly diminished in nearly all affected males as well as in a significant proportion of female patients. Therefore, a high mutation rate has been invoked to account for its high prevalence. However, as noted by Jacobs *et al.*,¹ there appears to be a dearth of new mutations. During the follow up of our 84 index patients, even after going back many generations, we have failed to detect any new mutations.² Similarly, in two other extensive surveys, no new mutations could be found.^{3,4} An alternative explanation for the lack of new mutations might be recurrent new mutations in the same pedigree. We could discount this latter possibility by showing linkage disequilibrium in a large fra(X) pedigree between the fra(X) gene and a closely linked polymorphic DNA marker. Thus, the fra(X) gene may be transmitted for many generations before becoming manifest. The realisation that new fra(X) mutations are very rare has far reaching implications for the counselling of (distant) relatives of fra(X) probands.

Material and methods

Fra(X) probands were ascertained as described previously.⁵ Genealogical studies were based on municipal documents and information from the families. DNA extracted from patients' blood samples was analysed for restriction fragment length polymorphisms, the CA dinucleotide repeat polymorphism at the *DXS548* locus, and for the CGG repeat in the *FMR-1* gene.^{2,5-7} The length of the CGG repeat in the normal range was also analysed by PCR.⁸

Results and discussion

SEGREGATION ANALYSIS IN A LARGE FRA(X) FAMILY

Five fra(X) probands with common ancestors in the early 18th century were ascertained in

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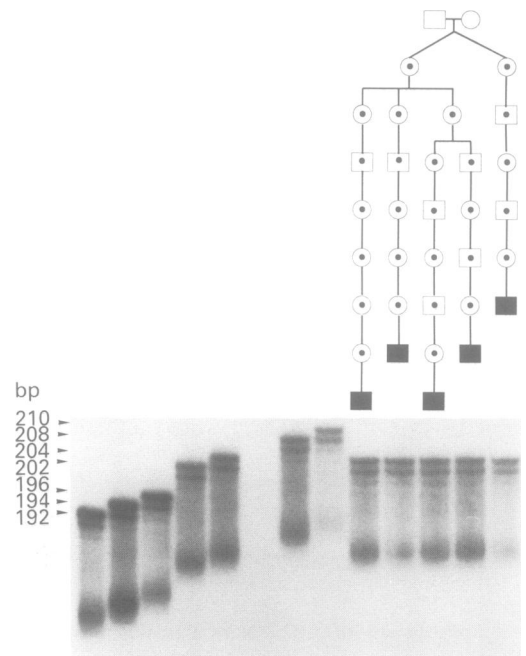


Figure 1 The polymorphic marker *DXS548* is in linkage disequilibrium with the *fra(X)* gene in an extended pedigree. Analysis of the CA repeat polymorphism at the *DXS548* locus in seven unrelated *fra(X)* patients (lanes 1-7) and in five *fra(X)* probands with common ancestors married in 1747 (lanes 9-12). With specific primers flanking the polymorphic CA repeat, a DNA fragment of 192 to 210 bp was synthesised by the polymerase chain reaction.⁸ The fragments were resolved on a 6% polyacrylamide gel. By labelling one of the primers with ³¹P the fragments could be visualised by autoradiography.

two different clinical genetics centres in The Netherlands. In all families there were two or more patients displaying the Martin-Bell phenotype and the chromosomal fragile site at Xq27.3. In two probands the fra(X) mutation was inherited from the maternal grandmother and in three probands the maternal grandfather had passed on the fra(X) mutation (fig 1).

The highly polymorphic DXS548 locus is located 140 000 bp proximal to the fra(X) gene locus on the X chromosome.⁶ DXS548 can be characterised by PCR analysis of the variable number of CA dinucleotide repeats at this locus. In agreement with our previous studies in a cohort of normal subjects and fra(X) patients from the United States,⁶ there is no

apparent linkage disequilibrium between the DXS548 marker and the fra(X) locus (see fig 1 for a subset of unrelated fra(X) patients from The Netherlands). However, when the five related probands were tested all showed exactly the same allele of 202 bp (fig 1). As this allele has a frequency of 15% in the population, the chances are less than 1 in 2000 that this result is fortuitous. It is obvious from the pedigree that the fra(X) gene can apparently pass through a number of male and female meioses before becoming manifest.

The fra(X) gene has been found to be a segment of unstable DNA^{9,10} caused by the expansion of a CGG trinucleotide repeat sequence in the 5' end of the FMR-1 gene.⁷ Normal subjects have between six and 46 copies of the repeat, while asymptomatic gene carriers may have 52 to over 200 copies of this repeat.⁸ Fra(X) patients are mosaics in which the repeat might be expanded up to thousands of repeats. Male transmission of the gene is not accompanied by large changes in repeat size, but in female meioses the repeat usually expands. However, this is not obligatory.^{3,4} It has been hypothesised by Sutherland *et al*¹¹ and shown by Fu *et al*⁸ that it is this plasticity of the fra(X) mutation that explains its variable clinical expression and its unusual genetics. Morton *et al*¹² have developed a model in which it is postulated that the expansion of the CGG repeat arises only on X chromosomes which have a larger than normal, but stable, CGG repeat. These alleles may be quite common and only when they increase further in length can the fra(X) syndrome develop in the next generations via female meioses. This model might also explain the inheritance seen in our pedigree in that the fra(X) has been transmitted many times without significant enough expansion to impede reproduction. Recently, Richards *et al*¹³ noted remarkable linkage disequilibrium between the fra(X) locus and two very closely linked CA repeats. The family presented here provides the basis for what these investigators observed in mostly Caucasian populations in the United States and Australia where only a few generations were traced.

DNA ANALYSIS IN A FAMILY WITH AN APPARENTLY ISOLATED FRA(X) CASE

The apparent lack of new mutations brings into question whether many more gene carriers might be identified through repeat analysis in any family. That this is likely to be the case is exemplified by our recent analysis of a family with a single patient (fig 2). Subject 1, a cousin of the patient (subject 11), wanted to know her carrier status before becoming pregnant. Linkage analysis with the closely linked flanking DNA markers indicated that the proband had inherited the grandpaternal X chromosome (fig 2, panel A). Direct analysis of the CGG repeat length by Southern blotting (fig 2, panel B) and PCR analysis (fig 2, panel C) showed in all daughters in the second generation an increased fragment of 200 bp, characteristic of the premutation in the fra(X) syn-

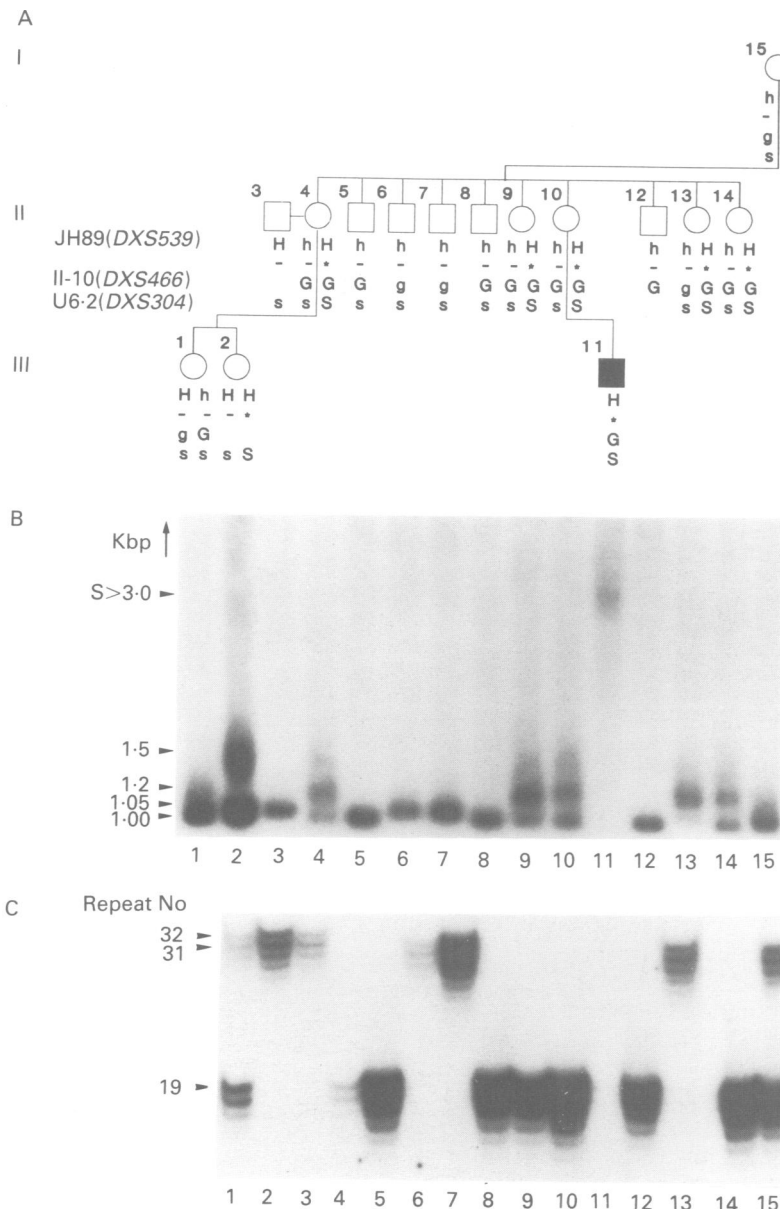


Figure 2 DNA linkage and direct mutation analysis in a family with a single fra(X) patient. Analysis of the polymorphic DNA markers DXS539 (Hh), DXS466 (Gg), and DXS304 (Ss) (upper panel A), and direct analysis of the CGG repeat by Southern blotting of PstI digests of the patient's genomic DNA (middle panel B), and by PCR with primers flanking the CGG repeat (lower panel C). In the pedigree the at risk allele is indicated by an asterisk. For subject 13, the Southern blot is not unequivocal as the signal of the normal X chromosome is not clearly distinguishable. This can be explained by the fact that this subject inherited the maternal X chromosome with the larger, but still normal, CGG repeat from her mother, the same allele as her two brothers (subjects 6 and 8). Owing to the inefficiency of the PCR for longer CGG inserts, the product of the expanded alleles in the gene carriers could not be shown. Only the consultand and her grandmother show heterozygosity for the normal sized alleles of the CGG repeat, confirming their non-carriership.

drome. This indicated that the grandfather must have been a 'normal transmitting male'. In the affected grandson (subject 11) a greatly expanded repeat of about 3000 bp in length was seen, while in his phenotypically normal cousin (subject 2), who had also inherited the grandpaternal X chromosome, a less pronounced expansion of the CGG repeat of 500 bp was seen. Of the females in this family, only the grandmother and the consultand did not carry the fra(X) gene.

Conclusion

The origin of the fra(X) mutation in the families described here as well as in virtually all other fra(X) families remains elusive. Neither has the conversion of a normal allele to a non-phenotypic premutation allele or to a full mutation been reported. However, the observation that the fra(X) chromosomes of different fra(X) families carry different alleles at the *DXS548* locus makes it unlikely that new mutations do not occur. Indeed, there is no a priori argument in favour of the absence of new mutations. It may take an as yet undetermined number of generations before the fra(X) mutation surfaces. In view of this latency of the fra(X) mutation, quite distant relatives of any fra(X) proband may be at risk for fra(X). Therefore, our data suggest that carrier detection by DNA analysis is indicated throughout

extended families identified through new probands, and that population based screening may be warranted.

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