POSTERS

NEW APPROACHES IN IDENTIFICATION AND MAPPING OF GENETIC SYNDROMES

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Analysis of Triplet Repeats of the FRAXA Locus Using a Novel Sequencing Procedure

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INTRODUCTION

Recent reports have shown that in normal alleles, the CGG repeat at the FRAXA locus is interrupted by one or two AGG and that abnormal alleles seem to be generated by expansion of pure CGG repeats at the 3' end [1].

It is therefore important to establish rapid, simple and low-cost sequencing procedures for determining not only the number of CGG repeats but also the presence of AGG triplets.

A one-lane sequencing procedure with PCR-amplified DNA, labelled at the 3' or 5' end with a single fluorochrome, has been recently developed in our laboratory [2, 3]. This methodology is particularly suitable for detecting mutations in family studies [4] and has been applied to the analysis of the sequence of triplet repeats at the FMR1 gene after PCR amplification, using one of the two primers fluorescently labelled at the 5' end.

MATERIALS AND METHODS

The sequence of the primers utilised in the PCR reaction is reported by Erster et al. [5]. The direct primer was fluorescently labelled at the 5' end with 5-(6)-carboxyfluorescein (Fluoreprime, Pharmacia) and synthesised using an ABI 391 PCR-Mate-EP DNA synthesiser. The PCR amplification was performed on 0,5 μg DNA in a 50-μl PCR buffer containing 10% DMSO, 10% glycerol, 25 pmol of the two primers, 0,3 m*M* dNTPs and 1 U Taq DNA polymerase.

Each sample was submitted to amplification on a Perkin Elmer 9600 PCR reactor, with the following cycling profile: 5 min at 95 °C, 30 cycles of 30 s at 97 °C, 1 min at 55 °C and 1 min at 72 °C, followed by a 10-min terminal extension at 72 °C. After precipitation with 5 *M* ammonium acetate, the pellet was dissolved in 80% (vol/vol) formamide

and aliquots were heated for 2 min at 90 °C or, alternatively, for sequence analysis, for 10 min at 110 °C. The samples were loaded on 6 or 8% PAGE sequencing gels and analysed using an ABI 373A automatic sequencer as previously described [2].

RESULTS

The analysis of the FMR1 alleles was carried out in control families as illustrated in figure 1, which shows the amplified fluorescent PCR products analysed in a 6% sequencing gel. The allele sizes and the pattern of transmission can be evaluated with precision, and alleles up to 400-500 bp could be amplified and detected (not shown).

The PCR product obtained from the amplification of the male alleles could be analysed for direct evaluation of the number of CGG triplet repeats and for the presence of AGG triplets which may interrupt the repeats. Figure 2 illustrates the analysis of the

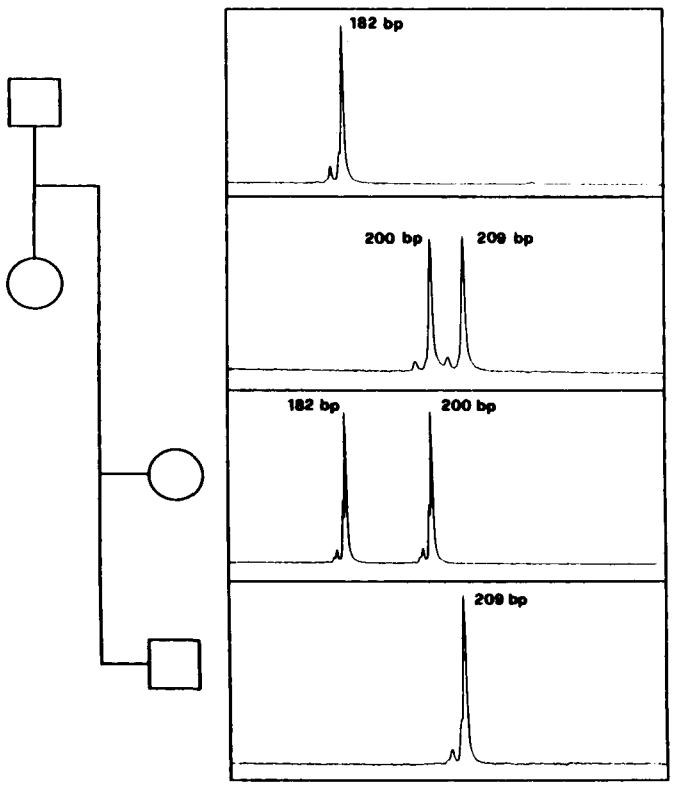

Fig. 1 - Analysis of the PCR products at the FRAXA locus, amplified from the DNA of a control family. The peaks correspond to the alleles containing different numbers of CGG triplets, as revealed on a 6% PAGE sequencing gel on an ABI 373A sequencer.

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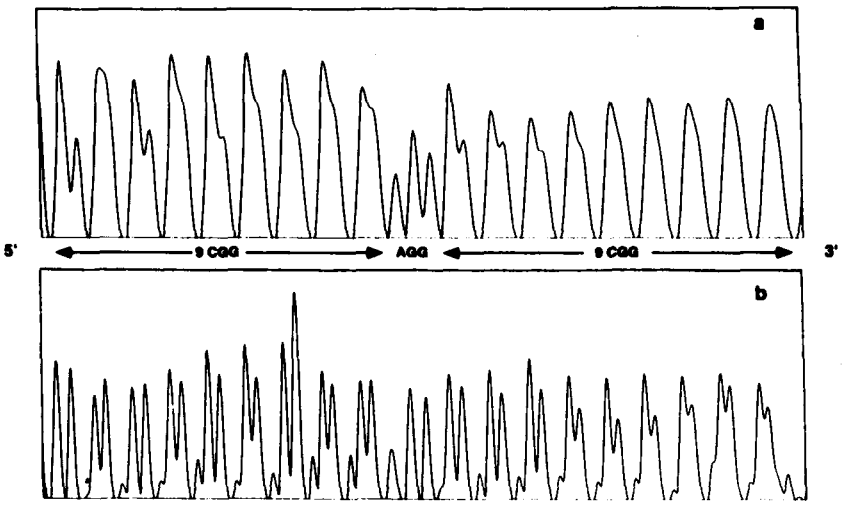

Fig. 2 - Sequence analysis of the CGG repeats of the father's 182-bp allele illustrade in Fig. 1, shoving the pattern of fragments obtained in a 6% PAGE sequencing gel (*a*) and an 8% gel (*b*) The 5' fluorescently labelled PCR product was degraded for 10 min at 110 °C in 80% formamide, loaded on to the sequencing gel and analysed using the single-lane sequencing software in an ABI 373A automatic DNA sequencer.

PCR product, after formamide degradation [2], from the father in the family reported in Figure 1, using the ABI sequencing software.

Figures 2a and b illustrate the portion of the sequence corresponding to the repeats analysed in 6 and 8% PAGE sequencing gels, respectively. Using this sequencing procedure, the CGG triplet can be visualised, in the 8% gel (Fig. 2b) as a small, sometimes accessory, peak (corresponding to the presence of a C) followed by two taller peaks (corresponding to the two Gs). These three peaks tend to be more poorly defined towards the 3' end of the PCR product. Under this analysis condition, the AGG triplet, interrupting the CGG repeats, gives rise to a defined pattern of three distinct peaks: one of intermediate size (correspondent to the presence of an A) followed by two higher peaks (corresponding to the two Gs). When the analysis is performed in a 6% gel (Fig. 2b), the CGG triplets are visualized as single large peaks, which are suggestive and may be related to the increasing 'compression' of the DNA due to the presence of the CGG triplets. The loss of this 'compression' corresponding to the AGG triplet, is more evident. Analysis of the same sequence using the standard sequencing procedures confirms this interpretation and the sequence of this DNA portion (not shown).

DISCUSSION

In the fragile X syndrome, the CGG repeat at the FRAXA locus may be interrupted by one or more AGGs [1] and it has been shown that the region containing the AGGs is stable and the instability is preferentially associated with alleles with more than 24 unin-

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terrupted CGGs, which predispose the allele to further expansion. It is therefore important to develop simple systems giving not only the allele size but also information on the DNA sequence.

The detection system we have described takes advantage of the one-lane sequencing procedure previously developed in our laboratory [2, 3], which has been further simplified for this specific application. The procedure does not utilise radioactive material and can differentiate alleles very accurately, since the analysis is performed on a sequencing gel. Furthermore, this procedure allows the direct visualization for the male alleles of CGG repeats and the interrupting AGG triplets. The analysis can be performed on any automatic fluorescent DNA sequencer and, once the PCR product is available, it requires only a few minutes of chemical degradation, with non-toxic reagents. This analysis provides information about the DNA sequence together with the allele size, which can be visualised in parallel lanes of the same gel.

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