SHORT COMMUNICATION

Direct analysis of the FMR-1 gene provides an explanation for an exceptional case of a fragile X negative, mentally retarded male in a fragile X family

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Received 1 June 1992.
Revised version accepted
23 July 1992.

Fragile X positive males with mental retardation have been shown by many groups to have an insertion or amplification of a trinucleotide CGG repeat at the fragile X site.¹⁻⁵ However, in some of these reports there have been males who appeared to be different in that they were cytogenetically fragile X negative but apparently had an insertion of DNA material and were mentally retarded. If indeed the amplification of the CGG repeat and cytogenetic expression of the fragile X site were one and the same, these males would seem to be exceptional cases. We previously reported on one such male^{5,6} who was part of an extended fragile X family (subject IV.9, fig 1). Although he had been studied cytogenetically three times under multiple media conditions, he was consistently negative. His IQ was 56 and he had always been in special education classes. Linkage analysis had indicated that he was likely to be carrying the same chromosome present in his cousins and other distant relatives affected with the fragile X syndrome. Jacobs,⁷ in an editorial on the fragile X syndrome, thought that this male could be

explained as "carrying a small mutation that is quite independent of his phenotype".

Because of the inconsistent presentation of mental retardation, apparent insertion of material at the fragile X site, and the lack of fragile X expression cytogenetically, we have studied this male further. The previously reported study involved using the available filters from the previous linkage studies.⁶ Following the development of other means of testing for the fragile X mutation involving a double digestion using *EcoRI* and *EagI*,⁸ we made fresh filters and probed them with both pOX1.9⁵ and StB12.3.⁸ The results for the latter are shown in fig 2. Probe pOX1.9 gave an identical pattern. As can be seen, the male IV.9 has a complex pattern which has been termed 'mosaic'.⁹ He clearly has a heterogeneous band indicative of an insertion or amplification of the trinucleotide CGG repeat segment at the

Figure 1 Pedigree of K:1000. All males who are cytogenetically fragile X positive have mental retardation.

Figure 2 Direct analysis of the fragile X mutation in K:1000. Southern filter of genomic DNA digested with *EagI* and *EcoRI* and probed with probe StB12.3. The lower arrow indicates a band of 2.8 kb while the upper arrow indicates a band size of 5.2 kb.

FMR-1 gene locus as well as a 'smear' indicative of methylation of some of his chromosomes. The latter has been shown also to be indicative of an affected male status.^{1-6,8,9} Both bands show an increase of 700 to 800 bp within FMR-1. Furthermore, two of his brothers who previously appeared to have a normal band using *Bgl*II filters,⁶ clearly have a pattern associated with transmitting males,^{8,9} with IV.6 also exhibiting some somatic instability. One brother, IV.6, graduated from high school and is at present serving in the military forces, while the other, IV.7, is progressing through high school at a normal rate. Neither one is classified as having learning disabilities or mild mental retardation.

These recent findings using a double digestion and direct analysis with probes pOX1.9 or StB12.3 clearly indicate the importance of reassessing all family members of fragile X families in order to determine the proper diagnosis of males with mental retardation and the detection of transmitting males. Subject IV.9 in this family definitely has the phenotype of fragile X and the phenotype is probably the result of a mutation similar to that seen in the majority of other fragile X males. At this point it is difficult to explain the lack of cytogenetic fragile X expression in his cells if indeed the amplification of the trinucleotide CGG repeat and the fragile X site are one and the same. It is possible that the amplification in FMR-1 is relatively small (0.7 to 0.8 kb) in our patient compared to other fragile X affected males where the amplification is often greater than

2 kb. Perhaps fragile X cytogenetic expression occurs more readily if the amplification is beyond 1 to 2 kb. It is likely that more detailed studies of males similar to our patient, perhaps using other tissues, will lead to a better understanding of the relationship between the cytogenetic findings and the molecular nature of the fragile X syndrome.

The authors would like to thank Drs Kay Davies and J-L Mandel for the probes pOX1.9 and StB12.3, respectively, and Judy Farmer for secretarial assistance. This work was supported in part by a grant from the South Carolina Department of Mental Retardation.

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