Acta Genet Med Gemellol 45: 295-297 (1996)

FRAXA and FRAXE: New Tools for the Diagnosis of Mental Retardation

A. Murgia, C. Vinanzi, R. Polli, L. Artifoni, F. Zacchello

Dipartimento di Pediatria dell'Università di Padova, Italia

INTRODUCTION

In the era of prevention and early diagnosis, mental retardation (MR) represents one of the most important challenges to modern medicine. Much needs to be done to restrict the number of different forms of this vast category of chronic handicaps for which accurate diagnoses are not yet available. The goal is to reduce the social burden and provide better care for patients and families.

The identification and characterisation of the molecular mechanisms which silence the FMR1 gene and which are responsible, in the majority of cases, for the fragile X syndrome (FRAXA) [1-4], the leading known cause of inherited mental retardation, led to the discovery of an extremely important new class of mutations: "dynamic mutations". These are highly unstable interspersed repeats, located close to or within genes, which show a strong tendency to expand. This discovery has raised the possibility for direct molecular diagnosis of FRAXA and several other diseases based on the same molecular mechanism, including a different form of MR associated with a fragile site in Xq28, named FRAXE [5].

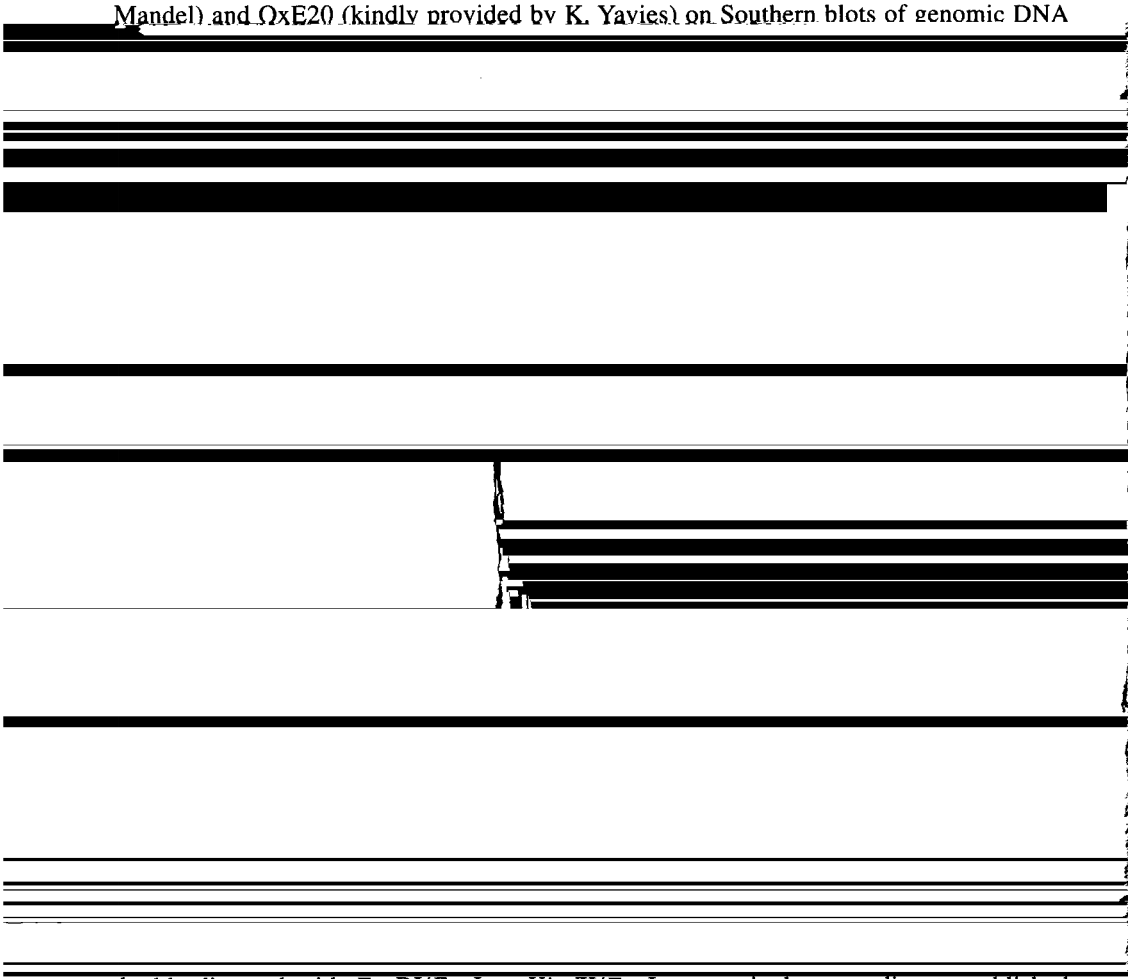
With these tools, we have started to study the structural characteristics and pattern of transmission of these mutations in a population of mentally retarded individuals mainly coming from north-eastern Italy. The aims of our study were (*a*) to establish the true incidence of FRAXA and FRAXE full mutations as a cause of mental retardation in our population, and (*b*) to re-evaluate families in which at least one individual had a cytogenetic fra(X) diagnosis, in order to identify mosaicisms and premutations that could not be identified cytogenetically, and to establish the carrier status of relatives of affected individuals.

MATERIALS AND METHODS

The population studied comprised: (*a*) 5 cytogenetically confirmed fra(X) families (n = 35); (*b*) 109 unrelated individuals with different degrees of MR of unknown cause,

mainly coming from north-east Italy, and (*c*) 10 relatives of newly identified affected FRAXA or FRAXE patients.

Molecular analysis for the detection of amplifications and abnormal methylation of the regions of $(CGG)_n$ triplet repeats associated with the fragile sites in Xq27.3 (FRAXA) or Xq28 (FRAXE) was undertaken using the probes StB12.3 (a gift of J.L. Mandel) and ΩxE20 (kindly provided by K. Yavies) on Southern blots of genomic DNA



double-digested with *Eco*RI/*Eag*I or *Hind*II/*Eag*I, respectively, according to published protocols [6].

RESULTS AND DISCUSSION

5 FRAXA full mutations (4.5%) were detected in a population of 109 unrelated individuals with MR of unknown origin. 46 individuals from 8 fragile X families were examined: 35 from families previously diagnosed as fra(X), and 11 from families identified in the course of our screening. Among the 17 phenotypically normal subjects at risk of transmitting an altered FMR1 gene, we identified 4 mutations (1 full and 3 premutations), while 13 individuals were definitely diagnosed as unaffected.

A total of 29 structural alterations of the FMR1 gene were detected: 11 premutations and 18 full mutations, 6 (33%) of the latter being mosaics, characterised by the co-pres-

REFERENCES

1. Kremer EJ, Pritchard M, Lynch M, Yu S, Holman K, Baker E, Schlessinger D, Sutherland GR, Richards RI: Mapping of DNA instability at the fragile X to a trinucleotide repeat sequence p(CCG)n. Science 1991; 252: 1711-1714.
2. Oberlè I, Rousseau F, Heitz D, Kretz C, Devys D, Hanauer A, Boue J, Bertheas MF, Mandel JF: Instability of a 550-base pair DNA segment and abnormal methylation in fragile X syndrome. Science 1991; 252: 1097-1102.
3. Verkerk AJMH, Pieretti M, Sutcliffe JS, Fu Y, Kuhl DPA, Pizzuti A, Riener O, Richards S, Victoria MF, Zhang F, Eussen BE, van Ommen GJB, Blonden LAJ, Riggins GJ, Chastain JL, Kunst CB, Galjaard H, Caskey CT, Nelson DL, Oostra BA, Warren ST: Identification of a gene (FMR-1) containing a CGG repeat coincident with a fragile X breakpoint cluster region exhibiting length variation in fragile X syndrome. Cell 1991; 65: 905-914.
4. Yu S, Pritchard M, Kremer E, Lynch M, Nacarrow J, Baker E, Holmann K, Mulley JC, Warren ST, Schlessinger D, Sutherland GR, Richards RI: Fragile X phenotype characterized by an unstable region of DNA. Science 1991; 252: 1179-1181.
5. Knight SJL, Voelckel MA, Hirst MC, Flannery AV, Moncla A, Davies KE: Triplet repeat expansion at the FRAXE locus and X-linked mild mental handicap. Am Hum Genet 1994; 55: 81-86.
6. Rousseau F, Heitz D, Biancalana V, Oberlè I, Mandel JL: On some technical aspects of direct DNA diagnosis of the fragile X sydrome. Am J Med Genet 1992;43: 197-207.

Correspondence: A. Murgia, Dipartimento di Pediatria dell'Università di Padova, Via Giustiniani 3, 35128 Padova, Italy.