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

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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 mosaicism 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 \((\text{CGG})_n\) triplet repeats associated with the fragile sites in \(\text{Xq}27.3\) (FRAXA) or \(\text{Xq}28\) (FRAXE) was undertaken using the probes \(\text{StB12.3}\) (a gift of J.L. Mandel) and \(\text{OXE20}\) (kindly provided by K. Yavies) on Southern blots of genomic DNA double-digested with \(\text{EcoRl/\text{Eagl}}\) or \(\text{HindWEagl}\), 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 \(\text{FMR1}\) 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 \(\text{FMR1}\) gene were detected: 11 premutations and 18 full mutations, 6 (33%) of the latter being mosaics, characterised by the co-presence of pre and full mutations. In 2 cases, the mosaic pattern included normal-allele band sizes, which we interpret as demonstrating complete reversion of the inherited mutation.

1 (0.9%) of 105 unrelated mentally retarded individuals tested was identified as carrying a full FRAXE mutation. This mutation was detected following \(\text{Hindlll}\) digestion of genomic DNA, and showed a pattern of marked expansion and somatic heterogeneity of the band (difference ranging between 1.6 and 0.8 kb). The unlikely possibility that this was a rare polymorphism detected by the probe/enzyme combination \(\text{OXE20/\text{Hindlll}}\), was excluded by using the \(\text{BamHI}\) restriction enzyme, which confirmed the alteration.

Double digestions with \(\text{Hindill}\) and the methylation-sensitive endonucleases \(\text{BsshII}\) and \(\text{EagI}\) clearly demonstrated complete hypermethylation of the region, compatible with the inactivation of a nearby gene.

Thus, we found the incidence of FRAXA full mutations in our population of mentally retarded individuals to be about 4.5%, while the frequency of FRAXE seems much lower.

We are currently studying a larger population to confirm the incidence of these mutations, and are extending the molecular analysis to a large sample of the general population to assess the distribution of normal and premutated alleles.

Molecular diagnosis to detect fragile X mutations has enabled us to give precise and definite information concerning their genetic status to members of several FRAXA families. Such information can significantly improve the quality of genetic counselling and ultimately the quality of care provided to mentally retarded patients and their relatives. The recent discovery of this previously unrecognised class of mutations represents both a crucial step toward the final goal of improving the diagnosis of MR, and contributes tremendously to a better understanding of the molecular mechanisms of inherited diseases.
REFERENCES


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