Annotations

Cloning of the gene for the fragile X syndrome: implications for the clinical geneticist

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As the commonest inherited cause of mental retardation, the fragile X syndrome represents a substantial workload for the clinical geneticist. This situation is compounded by its anomalies of inheritance and the limitations of cytogenetic demonstration of the fragile site at Xq27.3, which has a low sensitivity for asymptomatic carrier detection, an incomplete reliability for prenatal diagnosis, and logistical problems of cost and manpower for selected population screening. The recent cloning of the gene for the fragile X syndrome and the ease of demonstration of molecular pathology should transform this situation.¹⁻⁴

The gene for the fragile X syndrome (fragile X mental retardation-1, FMR-1)

The exons for FMR-1 span over 80 kb and encode a messenger RNA of 4.8 kb which is expressed in human brain, lymphocytes, and placenta.¹ The predicted protein has no significant homologies with known proteins, although the DNA sequence has some similarities to the human androgen receptor and to protamines and histones of different organisms. One striking feature of the FMR-1 sequence is a lengthy CGG trinucleotide repeat within the first exon which would encode a run of arginine residues. Normal males show minor length polymorphism in this region with 15 to 65 copies of the triplet (mean 40) and, in contrast to the situation in the fragile X syndrome, this length variation shows stable codominant mendelian inheritance.⁵ Disease pathogenesis is a multistep process involving length mutations of the CGG repeat and aberrant methylation of the CpG island adjacent to FMR-1.

FMR-1 in mentally retarded males with the fragile X syndrome

In affected males with the fragile X syndrome, the size of the DNA fragment corresponding to the CGG repeat is usually markedly increased.¹⁻⁶ The size of the normal fragment depends upon the probe and restriction enzyme combination but an increase of 1 to 4 kb (mean 1.5 to 2.5 kb) in affected males is usual with absence of the fragment of normal size. Furthermore, affected males often show multiple discrete larger bands (which decrease in intensity as the number of bands increases) or a faint smear of larger fragments which rarely (2 of 44 patients,² 3 of 111 patients⁶) can be associated with a band of normal size (presumably reflecting somatic mosaicism with a normal cell line). These additional bands or smears are believed to reflect further length mutations within somatic tissues and hence mutational instability in this region. This is also apparent in family studies where affected sibs commonly have different length mutation patterns. The GC richness of this segment interferes with PCR amplification and cloning and at the present time it is unclear if mutation is the result of variable amplification of the CGG repeat or whether an insertional event(s) could be involved. Exceptional patients have been described with a normal pattern on DNA analysis of this region,^{2,6} but several of these have been clinically atypical (for example, males with high levels of fragile X expression but no mental handicap and sporadic males with mental handicap but low or inconsistent fragile X expression) and thus the overall sensitivity of DNA analysis of this type for detection of the fragile X syndrome is high but possibly not 100%. In contrast, the changes seen in fragile X families have not been observed in over 250 normal subjects and a specificity of 100% is likely.¹⁻⁶

On the basis of breakpoints in somatic cell hybrids and in situ hybridisation experiments, the fragile site as seen cytogenetically appears to coincide with the CGG repeat. Mechanisms to account for the association of cytogenetic expression with excess

or deficiency of thymidine in the culture medium have been advanced,⁵ but the situation may be more complicated as rare fragile X positive patients do not show the characteristic CGG length mutations (see above).

FMR-1 in normal transmitting males

Normal transmitting males also show length mutations of the CGG repeat but in contrast to the affected males usually only a single band is seen (rather than multiple discrete bands or a smear pattern) and the increase in size is 150 to 500 bp (rather than 1 to 4 kb). In family studies these larger fragments were inherited by all daughters of normal transmitting males, either unchanged or with an increase or decrease in size of 200 to 400 bp, but the fragile X positive subjects in the next generation had much larger fragments with size and pattern variation. Thus, length mutation in the CGG repeat can occur at transmission by a male or by a female (or within somatic tissues). The CpG island associated with FMR-1 is usually unmethylated in normal transmitting males (and in normal males) in contrast to affected males where it is usually methylated.²

FMR-1 in carrier females

In almost all obligate carrier females an abnormal band is observed on DNA analysis regardless of their ability to express the fragile site on cytogenetic analysis.² Daughters of normal transmitting males have two bands, one normal and one 150 to 500 bp larger than normal, whereas females with fragile X expression have larger bands or a smear of fragments in addition to the normal band. Thus, while fragments with only a small increase in size are associated with a lack of clinical abnormality and with a low level or no fragile X expression in males and females, the reverse does not hold in females. Females with large fragments or smears in addition to the normal band may or may not express the fragile site and may or may not be mentally handicapped. A carrier mother with a large fragment may have affected sons with smaller or larger fragments.

Application of FMR-1 for carrier detection

Daughters of normal transmitting males usually have no or low levels of fragile X expression and in other obligate carriers only one half are cytogenetically positive. Virtually all obligate carriers studied so far have had detectable length mutations and molecular analysis would appear to have a clear advantage over cytogenetic analysis for carrier detection. This advantage in terms of sensitivity is also enhanced by the reduced labour and cost involved in molecular diagnosis of carrier status. Potential difficulties are

envisaged for carrier females with smear patterns and with gonadal mosaicism. The carrier females all have the normal sized fragment, representing the normal X, and, if the additional smear is faint, could be misdiagnosed as normal. This could be circumvented by the concurrent application of linkage analysis (see below). Gonadal mosaicism might be of relevance for counselling of the mother of an apparently sporadic patient. In such a case, even if the mother is negative on DNA analysis it would be prudent in the present state of knowledge to counsel that the risk to a future pregnancy is sufficiently high to merit consideration of prenatal diagnosis.

Direct mutational analysis can be supplemented in the molecular laboratory by linkage analysis in suitable families. Highly polymorphic AC microsatellite repeats which can be rapidly typed using PCR amplification are now available 150 kb proximal (*DXS548*¹) and 10 kb distal (*FRAXAC2*⁷) to the CGG repeat. Linkage disequilibrium is apparent across the CGG repeat⁷ and this helps to exclude this site as a recombination hotspot in this region of the X chromosome which is known for its generally high rate of recombination. So far no recombinants have been identified for either of these flanking polymorphisms and further family studies will define their recombination rates for genetic counselling purposes.

Application of FMR-1 for prenatal diagnosis

Multiple prenatal diagnoses have been successfully accomplished for the fragile X syndrome using cytogenetic analysis but the procedure is technically highly specialised and has incomplete reliability. Molecular analysis of chorionic villus samples should offer a practical and reliable alternative. These samples could be analysed by PCR for sexing and linkage using the flanking markers described in the previous section and also analysed by Southern analysis for length mutations in FMR-1. The latter is perhaps the most definitive test but might be subject to misinterpretation in the uncommon affected patient who has a normal band and a very faint smear of higher molecular weight fragments. This approach might well need cytogenetic confirmation until sufficient experience had been accumulated to determine its comparative reliability in clinical practice.

Application of FMR-1 for selected population screening

Most families with fragile X syndrome are currently unknown to the Regional Genetics Centres as routine screening of entire mentally handicapped populations has not been a practical proposition. As indicated earlier, male and female carriers (whether

clinically normal or abnormal and whether cytogenetically positive or not) can be readily detected on Southern analysis of the CGG repeat region of FMR-1. This test appears to be both highly sensitive and highly specific and given the value of early diagnosis for genetic counselling purposes the next step is to consider selected population screening.

Initially such screening would probably focus on mentally retarded males and females but if, as predicted by one hypothesis, the frequency of normal transmitting males is as high as the frequency of affected males, then it might be necessary to consider whole population screening for male and female carrier status for this condition.

Other applications

In addition to improved carrier detection and early prenatal diagnosis the families will also hope that cloning of FMR-1 might lead to treatment. Further characterisation of the protein product and determination of its mode of action are the next steps in this process and will be assisted by its conservation in other species. Furthermore, an improved understanding of the mechanism of mutation and the role of methylation in this condition might lead to prospects for prevention of development of the full phenotype.

Concluding comments

The cloning of FMR-1 is of major importance for the clinical geneticist in view of its predicted role in

carrier detection, prenatal diagnosis, and population screening for the fragile X syndrome. It also has a wider importance as it is the first illustration of a new type of human mutation. A similar type of mutation has since been reported in the androgen receptor gene in patients with X linked spinal and bulbar muscular atrophy⁸ and it has been postulated that mutational instability, as seen in the fragile X syndrome, might account for a wide variety of previously unexplained genetic phenomena including variable expression, anticipation, and incomplete penetrance.⁹

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