Editorial

The fragile X syndrome

It is clear from the number of articles on the fragile X syndrome in this issue that the recent identification of probes detecting the sequence at Xq27.3 that is altered in this disease, and the recognition of a gene, FMR-1, whose functional disturbance is intimately concerned with the affected state, has reawakened the interest of the human genetics community in this fascinating syndrome.

The timing of the Fifth International Symposium on X linked Mental Retardation in Strasbourg on 12 to 16 August proved very propitious. While the meeting covered all aspects of the fra(X) syndrome and other X linked mental retardation syndromes, the focus of the meeting was, understandably, on the recent molecular advances in fra(X). This aspect of the meeting is summarised in this issue by Dr Tessa Webb. While it is clear that the availability of the new probes has enormously simplified the accurate diagnosis of affected subjects, there are still a very large number of unanswered questions. The fra(X) mutation appears to be an increase in the number of CGG repeats present in one exon of the FMR-1 gene. Normal X chromosomes contain variable, but apparently stable, numbers of these repeats ranging from 15 to about 50. When the number of repeats increases to between 70 and 200 the subject is said to have a premutation or small insert. The mechanism by and the frequency with which this change from normality occurs is not known. No example of a mutation from normal to a small insert has yet been found, and it may be that the mutation is a series of small amplifications rather than a single event. The small insert appears to have a tendency to increase in size, especially when passed through a female, until it is greater than about 600 kb (that is, 200 copies) when it becomes methylated. Such large methylated inserts are somatically unstable and often appear as smears on Southern blots. It is the presence of the large methylated insert that is associ-

ated with the disease phenotype. As all families studied with this mutation have been ascertained through one or more affected subjects we have no data on the frequency with which a small insert is amplified to a large one.

There is a surprisingly good correlation between the size of the insert, the cytogenetic marker, and the phenotype, if subjects wrongly classified as fra(X) positive on the basis of a low number of fra(X) positive cells are disregarded. Thus, virtually all males with a large insert are fra(X) positive and retarded while all males with a small insert are fra(X) negative and phenotypically normal. The situation in females is not quite so straightforward. While all females with a small insert are fra(X) negative and phenotypically normal, those with a large insert may be fra(X) positive or negative and mentally impaired or normal. Neither the presence of the large insert nor the cytogenetic marker predict the mental status of a female with certainty. However, a rigorous reassessment of both the mental and cytogenetic status of women with a large insert might well resolve this problem.

All daughters of males with a small insert have a small insert while all carrier children of females with a large insert also have a large insert, there being only one reported case of a mother with a large insert having a child with a small insert. Offspring of females who carry the small insert can have either a small or large insert; any particular female with a small insert tends to have either only children with a large insert or only children with a small insert, there being very few mixed sibships with respect to insert size. It is clearly of both practical and theoretical importance to try to differentiate between females with a small insert who are likely to produce only children with a small insert and those at risk of producing children with a large insert. There are some preliminary data suggesting that the difference

between such women may depend on the size of their small insert.

It is evident both from previously published papers and those in this issue that the current crop of molecular probes has solved the problem of classifying subjects in fra(X) families; affected males have a large insert, females with a large insert are the only ones at risk of mental impairment, transmitting males have a small insert, and carrier females have either a small or a large insert. While the present situation is a great improvement on the recent past, where diagnosis was dependent on a cytogenetic marker and linked probes, it is not without problems. Firstly, there seems to be a small number of families in which X linked MR and apparently the same fragile site at Xq27.3 are cosegregating, but who have no recognisable molecular change. Secondly, as the mutation to a small insert is rare yet the large insert is common, there must be many subjects in the population carrying a small insert. Indeed, at the Strasbourg meeting, at least three pedigrees were described in which random spouses in fra(X) families were found to carry a small insert, and it was suggested that the frequency of small inserts in the population might be as high as several percent. It is therefore important to test all spouses in fra(X) pedigrees and also to realise that there may be carriers of a small mutation who are not at any immediate risk of producing an affected offspring. Such people might best be regarded as carrying a polymorphism rather than a premutation. The counselling of such persons is going to pose a problem for clinical geneticists. Indeed, many subjects currently thought to be exceptional may be explained by the presence of an unexpected small mutation. The exceptional subject in family F1 in the paper by Hirst *et al* in this issue is likely to be carrying a small mutation that is quite independent of his phenotype.

There seems little doubt that investigation of families already known to carry the fra(X) mutation is most accurately and economically carried out by molecular analysis of the insert. However, there is clearly still a place for linked probes providing they are both very polymorphic and very closely linked. The paper by Richards *et al* in this issue describes

just such a system. The authors have isolated two CA probes which immediately flank the fra(X) site. They are in linkage disequilibrium with one another so in practice only one need be used. Richards *et al* make a very compelling argument for the use of such linked probes in prenatal diagnosis before further studies with the molecular probe. Furthermore, in the small number of families who have mental retardation, a fragile site at Xq27.3, but no insert, linked probes analysable by PCR technology are still the fastest and most reliable technique for prenatal diagnosis.

Finally, what of the cytogenetic marker that was the key that unlocked the fra(X) syndrome? The molecular studies have, as yet, shed little light on the nature of the cytogenetic lesion, save to say it is associated with a large insert. In spite of the recent advances, the cytogenetic marker still has a very honourable role to play in the diagnosis of the syndrome. Population screening, if carried out for the fra(X) alone, is done most efficiently and effectively by molecular techniques. However, in the diagnostic laboratory screening with molecular probes will leave undiagnosed all those subjects with chromosome abnormalities other than the fra(X). As the cytogenetic test is reliable for virtually all males and for the great majority of, but not all, affected females, the most efficient and cost effective methodology for diagnostic specimens would still seem to be a cytogenetic analysis, followed by molecular studies only when the fra(X) is seen or suspected. Techniques which enable the cytogeneticist to carry out fra(X) screening and prometaphase banding on the same specimen will facilitate the work of the diagnostic laboratory. Griffiths and Strachan describe such a technique in this issue. As a cytogeneticist, I am naturally delighted that the cytogenetic lesion still has such an important role to play in the diagnosis, if not the understanding, of the fra(X) syndrome.

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