Review article

Journal of Medical Genetics, 1984, 21, 84-91

The fragile X syndrome: the patients and their chromosomes

MIGUEL A DE ARCE† AND ANTHONY KEARNS‡

From *the Section of Human Genetics, Department of Pathology, Faculty of Medicine, University College, Earlsfort Terrace, Dublin 2; and ‡St Michael's House, Goatstown, Dublin 14, Ireland.

Summary We have reviewed recent publications, mostly from 1980 onwards, concerned with the problem of identifying patients with the fragile X chromosome and mental retardation, considering the two practical sides of the problem, that is, identification by their external appearance and by chromosomal studies. We conclude that this condition covers a large range of physical findings which occur in varying degrees in people with the chromosome marker. We have tried to clarify the existent criteria that have to be considered for an accurate cytogenetic diagnosis.

External appearance of fragile X patients

People possessing the fragile X in their cells provide difficulties in identification by their external appearance. Even among the mentally handicapped it is not easy to decide which cases warrant further investigation for the abnormality. One possibility is to investigate all cases from families where there is a pattern of X linked mental handicap. This has been the procedure in a number of investigations.† ¹ There are drawbacks, however, including the fact that the family history is not always available. Even if it is, compiling it is time consuming as a routine method. The other main possibility is to attempt to use clinical traits as a means of narrowing the field, as in the case of Down's syndrome. Of the many characteristics ascribed to the fragile X syndrome some are more useful than others for this purpose. The principal features associated with this anomaly are as follows.

Mental Handicap

The range of this with the fragile X is considerable, extending from the profound to the borderline both in hemizygotes and in some, but not all, heterozygotes.³ The only apparent homozygote⁴ is normal. Most cases, however, appear to lie in the moderate to severe categories. Any surveys of mentally handicapped populations must be considered with this wide range in mind. Some studies have confined themselves to particular segments of the mentally handicapped population, for instance, those with speech defects⁵ or macro-orchidism,⁶ ⁷ and the incidence of cases in these populations may not hold for mental handicap as a whole.

Macro-orchidism

Macro-orchidism or testicular enlargement has a well recognised association with the fragile X.⁶ Association with X linked mental retardation was noted by Escalante et al.⁸ In 1975 Turner and her colleagues,⁹ investigating a number of families in New South Wales in a search for a cause for their apparent childlessness, discovered affected males who had bilateral testicular enlargement. Similar findings in mental retardates were noted by Cantù et al.¹⁰ and by Ruvacaba et al.¹¹ Following Sutherland's report citing fragile X in cases of X linked retardation,¹² Turner and her colleagues¹³ recalled a number of their families with X linked retardation. Of 18 families, they found that eight had macro-orchidism and the fragile X. The other 10 had testes of normal size and lacked the fragile X. Studies since then¹⁴ ¹⁵ ²³ ²⁴ ²⁵ have shown that this association is a very common one, although not invariable. In many of these studies a proportion of males was found to possess the fragile X but not to show testicular enlargement.⁷ ¹⁵ ²³ ²⁵ Conversely macro-orchidism can also be present without manifestation of the fragile X.¹⁴ ¹⁹ ²⁴ ³⁶ ²⁷

The presence of macro-orchidism in patients with the fragile X before puberty is more difficult to

† Supported by the Medical Research Council of Ireland.
Received for publication 5 October 1983.
Accepted for publication 10 October 1983.
The fragile X syndrome: the patients and their chromosomes

establish. Mattei et al. quoted its absence in 15 prepubertal subjects. Prepubertal males included in other studies have been shown to have normal or borderline or enlarged tests. The manner of measurement of testicular size is another important variable. Two principal methods are used. The first of these involves measuring testicular width and height with calipers and calculating volume using the formula: $V = (\text{length}) \times (\text{width})^2 \times \pi/6$ as described by Cantú et al. The alternative method is to use a series of ellipsoids of graded size comparing them with the palpated size of the testes. The 90th centile of testicular volume is between 23 and 25 ml and this figure has been used as an upper limit for normal testicular size in a number of studies. Zachmann et al. calculated mean testicular volume to be 18.6 ± 4 ml for normal men between the ages of 19 and 20. Where testicular function (hormone levels, sperm count, testis morphology) has been investigated in these cases, the evidence has been that it is normal. This would be supported by studies where an affected male has apparently fathered children. The enlargement seems to be the result of increased fluid.

FACIES
A fairly typical facial appearance has been described in many cases where the fragile X has been identified. Its features are lengthening of the face, high forehead, hypoplasia of the middle third of the face, large mouth and thick lips, long upper middle incisors, large jaws and prominent chin, very large and often poorly formed ears, head circumference normal to slightly increased, and palate high and arched. These features are not all present in all the subjects and are, in that sense, non-specific. It has also been suggested that the finding of blue eyes in a number of subjects is representative more of the northern European origin of these people than of a feature of the syndrome. However, the occurrence of a number of these features in a subject gives them an appearance of being ‘cast in the same mould’ or of having a ‘familial resemblance’. It is, however, important to recollect that the facies may be normal in appearance.

SPEECH AND LANGUAGE
Many patients possessing the fragile X have speech defects and delayed speech. Howard Peebles et al. in 1979 investigated members of four families with linked mental retardation, one of which had the fragile X. In these a general language disability was found with weakness in the area of auditory reception, auditory sequential memory, grammatic closure, and visual closure. Strengths were in non-verbal areas indicating that these people processed information more successfully through visual and tactile modalities rather than through auditory ones. A further study by Howard-Peebles and Stoddard in 1979 on another family with three brothers with the fragile X showed significant strength in auditory reception, visual association, and verbal expression in contrast with the four previous families. The only weakness in the fifth family was auditory sequential memory and this was a weakness in all four of the other families. Comparison of the two fragile X families showed either strength in manual expression or weakness in auditory reception in contrast to the three non-marker X families. It was felt by the authors in these studies that the samples were insufficiently large to provide a guideline for the use of language testing as a method of detecting X linked mental retardation. The possibility existed, however, that larger studies might allow the differentiation of the fragile X handicap from other forms.

There have also been other studies which have shown language retardation. Fitzsimmons et al. investigated four patients and reported nothing definite in their speech pattern apart from a limited vocabulary and speech becoming indistinct when they were excited, as in other patients with mental retardation. Carpenter et al. found a mild articulation disability in each of their patients. Language skills, they felt, were commensurate with overall intellectual levels. In the five males, language form was superior to content and use and perseveration was noted. Herbst et al. in 1981 did not discover any general verbal disability or developmental apraxia in the affected males in their study. These males came from families both with and without the fragile X. They were also unable to note any differences in auditory reception and manual expression in the males with or without the marker. Jacobs et al. found most affected males to have a characteristically repetitive jocular form of speech, so much so that they were able tentatively to identify two subjects on the basis of speech alone. Rhoads in a letter to Pediatrics in 1982 describes having been able to make a diagnosis of fragile X on the basis of a telephone conversation with an adult. Jacobs et al. discuss a repetitive speech pattern which presented in all the men studied capable of sustained verbal expression and even among the more retarded capable of speaking only in single words or short phrases. Theobald and Hay showed males with the marker X to have a verbal IQ far in excess of their performance IQ. This did not agree with the findings of Lehrke or Deroover et al. although the latter two studies were concerned with X linked mental retardation in general, not specifically fragile X.
linked handicap. Lehrke had in fact suggested that one or more genes on the X chromosome related specifically to verbal function, hence the tendency for verbal dysfunctioning to occur in X-linked anomalies.

**Behaviour**

Not all descriptions of cases of patients displaying the fragile X show conclusive disturbances of behaviour. Mattei et al.\(^\text{28}\) described 15 families comprising 20 cases, 19 of whom were boys. In these, the principal behaviour characteristics were obtained from their parents and teachers and disturbed behaviour was apparently noted in all cases, although this is not elaborated on. Kakhönen et al.\(^\text{17}\) noticed no specific behaviour problems in their series of 12 males with the marker X. Fitzsimmons et al.\(^\text{18}\) found a cheerful attitude in the four males that they studied with the marker. Herbst et al.\(^\text{24}\) studied a series of patients from families both with and without the marker X and found them generally to be well mannered and pleasant with reasonably good independence skills. Jacobs et al.\(^\text{25}\) in nine patients with the marker X, described at least three of them as having been hyperactive in childhood. Two had been described as autistic. Almost all were shy initially and four were quite fearful. None became violent. All were friendly when comfortable. Autism was also found in four of 20 cases of fragile X\(^\text{37}\) and in eight of 30 in another series.\(^\text{38}\) Hyperactivity, anxiety, mood lability, and autistic features have been described elsewhere.\(^\text{38}\) Jacobs et al.\(^\text{25}\) described most of their 27 cases as happy, out-going, and, in their own way, communicative. Two of the patients studied by Martin and Bell\(^\text{84}\) in 1943 were described as having pronounced psychotic traits. A reinvestigation was done by Richards et al.\(^\text{41}\) in 1981 on members of this family and showed the presence of the fragile X. As can be seen from the foregoing, studies of behaviour in patients possessing the fragile X are limited so far and yield apparently no specific findings apart, possibly, from the connection with autism. Further more detailed investigation in this field is needed.

**Other**

In an effort to delineate a phenotype, other features have also been suggested as forming part of a fragile X syndrome. Increased birth weight was noted by Turner et al.\(^\text{18}\) However, this has not been a significant finding in other studies. Obesity has also been commented on by some workers.\(^\text{18}\)\(^\text{25}\) Again this has not been consistent. Short stature or increased occipitofrontal circumference have been seen in others,\(^\text{26}\) but their presence seems to be the exception rather than the rule.

**In Brief**

As can be seen from the above commentary, there are no pathognomonic features of the fragile X syndrome. However, running through the various studies are certain main findings, such as mental handicap of some degree, macro-orchidism, a group of facial characteristics, some or all of which may be present, large ears, and behavioural abnormalities.

Therefore, it seems at present that the condition covers a large, but nevertheless circumscribed, range of physical findings which occur in varying degrees in people with the chromosomal marker.

**Laboratory Diagnosis**

The detection of the fragile X in the laboratory is not a routine procedure. Basically it requires the growth of peripheral lymphocytes in a medium with low concentrations of folic acid, like TC 199.\(^\text{42}\) The picture that emerges in the case of a true positive resembles mosaicism; a proportion of the cells show the fragile X, seldom more than 50% with present techniques. The rest of the cells have a normal X.

The possibility of false positives arises because of our ignorance on two points. Firstly, we do not know if some normal people show the fragile X. Secondly, we do not know if some of the fragile X like chromosomes that are seen in some cells are the genuine product, or some autosomal (if only unbalanced cells are being studied), or an X chromosome with a gap that appeared for other unknown reasons. The possibility of false negatives arises because it could be difficult to analyse the number of cells required to obtain a high probability of finding a fragile X when it occurs with low frequency, and because we do not know, and therefore cannot control, all the factors that induce the fragile X to express itself in all cells.

**Fragile X in Normal People?**

This question has been approached in several ways. The most obvious approach is to analyse normal people. The first report of this type suggesting a positive answer came from Daker et al.\(^\text{43}\) Their two probands were two brothers with the fragile X in 15 and 8% cells, respectively, confirmed by banding. The nature of the medium in which the lymphocytes were grown was not stated, the daughters were not studied, and the intelligence of the probands was not quantified, although it appeared normal. Doubts on the genuineness of the report were
The fragile X syndrome: the patients and their chromosomes

cast by Hecht et al on at least two occasions, in a report on the Jerusalem Conference44 and in reply to a letter.45 They stressed the negative points made above and argued that Sutherland46 in a study on 1019 neonates had not found even one with the fragile X. Nevertheless, Sutherland himself mentioned in that paper that all that could be concluded from his study was that "the fragile X is much less common in the normal population than among mental retardates". Replying to the questions of Hecht and his colleagues, Daker47 defended his original observation by saying that "no matter how many neonates have been examined with negative results, our incontrovertible findings remain, and the simplest explanation for these is that the fragile X may occasionally be compatible with mental normality in the male". The matter appeared less doubtful when Hecht et al48 admitted that "...we have been impressed by an increasing number of reports of allegedly normal males with the fragile X... although such males must be very uncommon". Any future study to assess the incidence of fragile X chromosomes in the normal population should cover two possibilities; firstly that intellectually normal subjects with reasonably high frequencies of cells expressing the fragile X, as in Daker's probands, might be very rare and, secondly, that they might be more common, but have very low frequencies of cells expressing the fragile X, as found by Popovich et al.49 If this were the case, it would not be all that surprising that Sutherland did not find them among his neonates, since he looked at 50 cells per case. As we have speculated,50 with this relatively low count he could rule out with reasonable confidence only those cases with frequencies of expression over 6%.

Another way to approach the question of the existence of fragile X in the normal population is the study of families. In this way, Brøndum-Nielsen et al,61 Webb et al,52 and Fryns and Van den Berghe53 have shown strong evidence from pedigrees that apparently normal males must have passed the fragile X to their grandchildren but, unfortunately, in these three cases the fact was recognised posthumously. To obscure the situation further, when the proband was available, as in the case reported by Rhoads et al,40 this probable hemizygote of normal intelligence consistently failed to show the fragile X.

A third way to find if normal males carry the fragile X or not was proposed by Howard-Peebles,54 and that is to study the brothers of affected males when the mother is a known carrier. This approach is attractive because it is testable. In these families, 50% of the males should have the fragile X and be mentally retarded and 50% should be normal and not have the fragile X, if the hypothesis that the locus for fragile X linked mental retardation is at the fragile site Xq27 is true. In the hypothesis of no association between mental retardation and the presence of the fragile X, half of the non-retarded brothers should have the fragile X, as pointed out by Silverman et al.55

Unfortunately, there are very few families reported in which all members have been studied both psychologically and cytogenetically, but the data so far support the hypothesis that the locus for mental retardation in this syndrome is at the fragile site. It is perhaps because of this, and in spite of the scarcity of data and of the postulated but necessary existence of normal males with the fragile X, that Kaiser-McCaw et al66 emphatically underlined that there is no crossing over between the fragile site and the hypothetical locus for mental retardation in this syndrome. Nevertheless, if these two loci were distinct but very close, they would recombine very seldom, and this would explain the rarity of normal males carrying the fragile X and also suggest that more data from brothers should be gathered to demonstrate a low frequency of recombination, or before asserting the overlapping of both loci.

Two years later in 1982, Hecht et al57 sounded more cautious: "...we do not have the least idea whether the fragile site on the X is merely linked to an X linked locus for mental retardation".

When is a fragile X a fragile X?

It has been repeatedly observed that under the conditions required to induce the expression of the fragile X, the number of non-specific gaps and breaks occurring in nearly any chromosome in the complement increases.13 20 49 58 Little is known about the frequency with which these non-specific lesions occur and their distribution. Mattei et al59 studied this matter in over 1000 banded karyotypes and found about 6% breaks or gaps in the normal population, together with some kind of seasonal variability. Seven per 1000 of the lesions occurred on Xq27 and more on telomeric sites on other chromosomes of the C group, but, unfortunately, they did not specify their culture medium. From the work of Sutherland, we know that TC 199 does induce the expression of certain fragile sites on autosomes of the normal population, in particular on 6q26 and 10q23. These do not seem to have any phenotypic effect60 and can also be found in mental retardates with the fragile X.61 Nevertheless, it has been repeatedly suggested that their presence could lead to false fragile X positive diagnoses62 63 when looking at unbanded slides. Soudek and McGregor61 have found that in their material about 2% of these
fragile X-like chromosomes are in fact autosomes, as revealed after banding slides that had previously been considered, using orcein staining, to show the fragile X. This occurred both in mental retardates and in normal controls. Several recommendations have been made to minimise this problem. A very popular one is to establish a threshold for the frequency of fragile X-like chromosomes at 4%. Frequencies of fragile X-like chromosomes below this are supposed to be irrelevant for the positive diagnosis. It is assumed that this applies only to unbande slides. This criterion was originally suggested by Jacobs et al.28 and is obviously not unambiguous, so that borderline cases should be banded. For instance, Rhoads et al.60 considered the presence of the marker in 3% of cells to be of diagnostic value in view of a pedigree indicating an obligate carrier.

In view of the very many reports of mentally retarded patients who also display other characteristics of the fragile X syndrome, and who express the fragile X in less than 4% of their cells (see for instance Fryns and Van Den Berghe28; 14 out of the 37 males reported showed 4% fragile X or below), it would appear that this lower threshold is too high, particularly for carriers, and its only justification would be in cases where no banding is possible. Herbst et al.63 suggest a minimum threshold of expression of 1%, and Steinbach et al.68 have shown that, provided that non-specific lesions on the long arm of the X occur with a frequency comparable to the average frequency of such lesions in autosomes, the minimum threshold required for positive confirmation of a carrier is 0.7% in males and 1.5% in females. These figures refer to observations made on banded slides from cells grown in TC 199 and are surprisingly similar to the ones found by Mattei et al.69 as the background frequency in the normal population, in banded metaphases, from cells grown in unspecified medium.

A doctor’s dilemma

The early 1980s are witnessing a rush to entice the fragile X to express itself reliably in lymphocytes, fibroblasts, amniocytes, or fetal cells. The immediate aims appear to be respectively: to detect patients more accurately and easily, to detect carriers in order to provide them with the required genetic counselling, to detect the fragile X in utero, and, in the case of confirming its presence in the fetus, abortion.

To detect patients more accurately from lymphocytes one could try to increase their ‘visibility’ by physicochemical means once the slides are made, or the frequency with which the fragile sites express themselves by using biological means while the cells are in culture. On the first line, Zankl and Eberle64 have observed that orcein stained slices in phase contrast improve the ‘visibility’ of the fragile X. Harrison et al.65 have attributed the reduced frequency with which the fragile X is seen under light microscopy to the low resolving power inherent in that instrument; they were able to see more fragile Xs in the same patient with the scanning electron microscope than with the light microscope. It would appear that its typical aspect might be lost more often in G banded slides. In our experience, R banding gives a clearer picture, but the bromodeoxyuridine used to R band in the FPG or RBG techniques probably reduces the frequency of expression.66

The effect of 5-fluorodeoxyuridine (0.4 mol/l at the beginning of the culture) in enhancing the frequency of expression was reported simultaneously by Glover67 in America and Tommerup et al.68 in Europe. A month earlier (August 1981) Mattei et al.69 had reported a similar effect with the addition of 10 mg/l methotrexate 24 hours before harvesting. These results reveal some details of the biochemistry involved in the expression of the fragile site; methotrexate inhibits the action of dihydrofolate reductase, and fluorodeoxyuridine inhibits the action of thymidilate synthetase. Both enzymes control the transformation of dUMP to dTMP, one of four bases that constitute DNA, but the exact way in which these contribute to the expression of fragile sites remains obscure. From a practical point of view, they are nevertheless useful because they can induce the expression of fragile X in some patients who otherwise would not have shown it.

The demonstration of the fragile X in fibroblasts was reported by Jacky and Dill in 1980.70 They attributed the higher frequency in fibroblasts to the more gentle harvesting technique and to the use of Na-citrate as hypotonic treatment instead of KCl. They also felt that the degree of chromosome condensation is important in enhancing the frequency, and this was confirmed later by Barbi and Steinbach71 by analysis of prometaphases. Fontach72 has remarked that the frequency of metaphases with the fragile X is greater in early passages than in late passages. Like Mattei et al.69 she used MTX and also aminopterin for 24 hours before harvesting, but she used KCl as hypotonic treatment.

Generally speaking, it would appear therefore that the fragile X is expressed in fibroblasts more frequently than in lymphocytes, particularly if the cultures are manipulated in the ways described above. But expression of the fragile X in 100% of cells has not been achieved yet.

Amniocytes have also been cultured successfully.
The first report came from Jenkins et al using methods similar to those described for fibroblasts. Their report was immediately followed by a similar one, which also showed the fragile X in lymphocytes from an affected fetus, and by Schmidt et al in Europe. These and other authors have suggested another component to the problem, that is, genetic heterogeneity in the frequency of expression of the fragile X (some families appear to be easier than others in which to demonstrate the fragile X). Individual consistency in successive cultures has been noticed by Eberle et al.

All of this brings us now to the doctor’s dilemma, which could also be called the unknown quantity for the genetic counsellor. Prospective parents will require to know not only the probability of passing the fragile X to their children, but also the extent to which the children who receive it will be affected. This is a difficult problem since it involves not only the laws governing the transmission of the chromosome, but also those governing its expression, and these are less well understood.

In the case of male descendants, it is clear now that even low frequencies of expression of the fragile X are found in mildly to moderately retarded children, and it would appear that the degree of mental retardation is positively correlated with the frequency of expression of the fragile X, but what determines this correlation is still unknown. The expected 1 in 2 risk of a mentally retarded son can be predicted from an obligate carrier mother with reasonable confidence, particularly if there is a history of familial mental retardation, although the degree of mental retardation is at present impossible to predict.

The situation in female descendants is complicated by the fact that they have two X chromosomes in their cells. Very early in the embryonic life of the carrier, one of the two X chromosomes is inactivated in every cell, and it has been suggested that the selection of which one is inactivated is not always random. The number of cells that have an active fragile X and that constitute the nervous system, or those parts of it related to intelligence, might vary from person to person, and this could determine the degree of mental development of the carrier. This might explain the different degrees of intelligence found in obligatory carriers, most of whom are normal, but some of whom are dull or even severely retarded. This hypothesis also finds support in recent observations by Howell and McDermott and Uchida and Joyce, who found that in one severely retarded carrier the proportion of active fragile Xs was 80%, while in two mentally normal carriers the proportion was below 50%. In brief, the case of female progeny, the prospects are much better since many obligate carriers do not show mental retardation at all. Nevertheless, the possibility of some degree of mental retardation should be left open. It is not possible at present to quantify this risk exactly or to predict the degree of mental retardation.

It would appear to us that in this, as in other situations, Science must steer her course carefully among principles of uncertainty. While studying fetal blood, there appears to be no difficulty in determining the presence of the fragile X, but then, as Hecht et al have pointed out, “which of the fragile X male fetuses is destined to be retarded (and to what extent), and which is programmed to be of normal intelligence?”. And, conversely, in several cases as we have seen, family studies have pointed towards particular persons, perhaps fresh mutants, as carriers of the fragile X, but then either the corresponding mental retardation, or the fragile X itself, was not expressed.

Before systematic abortion of positive cases becomes the general practice, in spite of our present lack of understanding of the syndrome, we consider that more research should be done in the area of therapy; at least three groups have reported various degrees of amelioration in patients treated with folic acid. More research will show the type of patient that is likely to benefit most from this treatment, or could confirm the possibility that the fragile X syndrome may be more than one clinical entity, perhaps the heterogeneous result of different mutations of the same gene. This could explain the variability in presentation of this syndrome and in the response to treatment.

The authors wish to thank Dr Barbara Stokes for encouragement while preparing the manuscript, and the secretarial staff of St Michael’s House, Ballymun, for assistance in typing.

References

6 Sutherland GR, Ashforth PLC. X-linked mental retardation with macro-orchidism and the fragile site on Xq27 or 28. Hum Genet 1979;48:117-20.


The fragile X syndrome: the patients and their chromosomes


Correspondence and requests for reprints to Dr M A De Arce, Section of Human Genetics, Department of Pathology, Faculty of Medicine, University College, Earlsfort Terrace, Dublin 2, Ireland.