Fragile X testing in a diagnostic cytogenetics laboratory

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SUMMARY  Chromosome results obtained from 1012 patients referred with developmental delay without known cause within the three years 1985 to 1987 are reported. G banding analysis and assessment of 70 cells for fragile X gave abnormal results in 84 cases: fragile X in 31 patients and other abnormalities in 53 patients. A further 16 sibs expressing the fragile X were detected in family studies originating from the 31 index cases. This yield justifies continuation of procedures which detect both fragile X and subtle chromosomal abnormalities in these patients.

Work in the 1970s associated a form of X linked mental retardation with chromosome fragility at Xq27. Since then extensive clinical, cytogenetic, and molecular studies have been carried out in an attempt to characterise this syndrome. The fragile X syndrome is the second most common cytogenetically diagnosable abnormality among the mentally retarded, after Down's syndrome, and the most common transmissible cytogenetically diagnosable syndrome. The fragile X chromosome is estimated to have an incidence of between 1/2000 to 1/2500 in males and 1/1000 to 1/1250 in females. About one-third of heterozygous carriers are clinically affected. A wide range of IQ is observed in both males and females who express the fragile site. Some phenotypically normal males have transmitted the syndrome. The physical phenotype in both males and females is variable and may not be recognisable clinically. The genetic basis of the syndrome has not yet been identified and the cytogenetic finding of the fragile site at Xq27 remains the principal diagnostic criterion.

The recognition that the fragile X syndrome can be associated with developmental delay without other identifying clinical features has led to the acceptance of patients with developmental delay only for cytogenetic examination. The opportunity of preventing further cases by identifying carriers among the female relatives of those with the fragile X syndrome adds to the importance of diagnosis of index cases through clinical referral or by systematic screening of all children with delayed development.

This paper analyses experience over a three year period in a laboratory with an average of 1600 clinical referrals per year.

Methods and results

SOURCE OF THE DATA

Data were obtained from the records of the cytogenetics laboratory at the Royal Children’s Hospital for the three year period 1985 to 1987. Referrals to this laboratory come from throughout the states of Victoria and Tasmania from a total population of 4-5 million. They are referred by clinicians associated with public hospitals and in private practice, as well as various clinical units within the Royal Children’s Hospital. Cytogenetic assessment for the fragile X was carried out on all referrals where the clinical information supplied by the referring clinician included developmental delay.

CYTOGENETIC ANALYSIS

Cells were routinely cultured in the folic acid deficient medium 199 which permits expression of the fragile site Xq27. No special treatment was used to obtain prometaphase chromosomes, but the colchicine time was limited to one and a half hours. Cytogenetic analysis was carried out on G banded preparations at the 550 to 850 band level. It involved a count of 15 cells with karyotypic analysis of five cells, and examination of X chromosomes for the fragile site Xq27 in a further 55 cells.
The cytogenetics laboratory at the Royal Children's Hospital does not carry out prenatal diagnostic testing and has a large paediatric component in its work. Of 4953 subjects studied in the laboratory in the three year period, 1012 patients had a provisional diagnosis that necessitated examination for the fragile X. This is 20.4% of the total investigations. In the period 1985 to 1987 there was an increase in the annual total number of fragile X investigations (including family studies), from 299 to 519, representing an increase from 22% to 28% of the work load. This trend has continued in 1988.

PATIENTS EXAMINED FOR FRAGILE X

A total of 1012 patients was investigated and abnormal cytogenetic results were obtained in 8.3% (table 1); 3.1% of the patients had fragile X and 5.2% had other chromosomal abnormalities.

Probands examined for the fragile X were divided into two groups on the basis of the provisional diagnosis (table 1).

1. Developmental delay without major congenital abnormalities, but including patients with minor dysmorphic features suggestive of the fragile X, that is, patients in whom it might be assumed that the clinician had no specific diagnosis other than fragile X in mind (892 patients examined).
2. Developmental delay/mental retardation with congenital abnormalities, that is, patients fitting the more traditional reasons for chromosomal referral (120 patients examined).

FINDINGS IN PATIENTS WITH DEVELOPMENTAL DELAY/MENTAL RETARDATION PLUS CONGENITAL ABNORMALITIES

This group yielded nine abnormalities other than the fragile X. The frequency of 7.5% is in accord with general experience. The abnormalities detected included apparently balanced rearrangements (three); unbalanced chromosomal rearrangements (five) including marker chromosome (one), deletions (three), and duplication (one); and sex chromosome aneuploidies (one) (appendix).

Fragile X was detected in four patients who, on the basis of their provisional diagnosis, had been placed in the MR/CA group. These four patients were reassessed clinically and found to be either not apparently balanced rearrangements (12) including inherited rearrangements (six known) and de novo rearrangements (four); unbalanced chromosomal rearrangements (22) including ring chromosome (two), markers (three), deletions (eight), and duplications (nine); and sex chromosome aneuploidies (10) (for list of abnormalities see appendix).

Although these patients were all referred to the laboratory with a provisional diagnosis of developmental delay only, six patients were found to have significant physical abnormalities when examined by our clinical colleagues. Even though mild dysmorphism might be observed in a patient with developmental delay, it is frequently the developmental delay that prompts the referral for cytogenetic investigation.

The fragile site Xq27 was found in 27 subjects in 26 families not previously diagnosed, a detection rate of 3.0%.

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<table>
<thead>
<tr>
<th>Provisional diagnosis</th>
<th>Normal results</th>
<th>Abnormal results</th>
<th>Total investigations</th>
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<td></td>
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<td>Fragile X</td>
<td>Other abnormalities</td>
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<tr>
<td>Developmental delay</td>
<td>821</td>
<td>27 (3.0%)</td>
<td>44 (4.9%)</td>
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<td>MR/CA</td>
<td>107</td>
<td>4 (3.3%)</td>
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<tr>
<td>All referrals</td>
<td>928</td>
<td>31 (3.3%)</td>
<td>53 (5.2%)</td>
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<th></th>
<th>Male</th>
<th>Female</th>
<th>Total</th>
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<tr>
<td>Total investigations</td>
<td>516</td>
<td>486</td>
<td>1012</td>
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<tr>
<td>Fragile X positive</td>
<td>23 (4.5%)</td>
<td>8 (1.6%)</td>
<td>31 (3.0%)</td>
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<td>First degree relatives of index cases</td>
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<tr>
<td>Total investigations</td>
<td>15</td>
<td>41</td>
<td>56</td>
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<tr>
<td>Fragile X positive</td>
<td>5 (33.3%)</td>
<td>18 (43.9%)</td>
<td>23 (41.1%)</td>
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Fragile X is sufficiently high to contribute significantly to the value of testing patients with developmental delay only. We estimate that about one third of these other abnormalities would not be detectable without chromosome banding. In particular, plain staining would miss small deletions which are often associated with intellectual disability without dysmorphism. Thus, there is a need to use banded preparations for the analysis of these patients, quite apart from the value of banded preparations for fragile X detection.

Of the 31 index cases, 23 (74%) were male (table 2). This is a little above the expected ratio of three male:two female among affected subjects. A bias towards selection of more severely retarded patients for cytogenetic referral may explain the slight deficiency of fragile X positive females, as they are generally more mildly retarded.

In three of our patients an unbalanced chromosomal rearrangement has been inherited from a mildly retarded parent with the same unbalanced rearrangement. Another unbalanced rearrangement involving the X chromosome is present in a developmentally delayed male sib and so it is presumably inherited; both parents are phenotypically normal but were not studied cytogenetically. Parental studies were incomplete in four cases while all other unbalanced rearrangements were de novo. While an unbalanced rearrangement is considered causative of developmental delay, many of the other abnormalities detected are of a type that has been associated with a normal phenotype. They may be a coincidental finding, for example, with the balanced Robertsonian translocation, rob(13;14), but such a conclusion is more equivocal with inherited inversions. The incidence of de novo, apparently balanced rearrangements has been ascertained in surveys of the mentally retarded and of newborns. Warburton compared data from the two groups and concluded that the presence of a de novo, apparently balanced rearrangement is associated with an increased risk of mental retardation, with an odds ratio of 6-0 to 7-0. This suggests that the de novo, apparently balanced rearrangements seen in our sample are frequently, if not always, responsible for the retardation.

The detection of sex chromosome aneuploidy within this referral group is consistent with recently released longitudinal studies on children with sex chromosomal aneuploidies, which show that a high proportion of these children are found to have learning difficulties.

While follow up of the families of the 31 index cases with the fragile X is still incomplete, the identification of five secondary cases and 11 carriers among the sibs of these patients indicates the particular advantage in diagnosing subjects with the fragile X syndrome.
We wish to thank Professor D. M. Danks and Dr. M. Schmidt for critical reading of the manuscript.

References


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**APPENDIX** Abnormalities detected other than fragile X.

### Apparent balanced rearrangements

**Patients referred with developmental delay only**

- 46,XY,t(9;15)(q32;q15) pat
- 46,XY,t(7;9)(q32;q34) de novo
- 46,XY,t(2;11)(p23;p15) de novo
- 46,XX,inv(7)(q11q22) mat
- 46,XY,inv(2)(p11q13) mat
- 46,XY,inv(12)(q12q21) pat
- 46,XY,t(1;16)(q12;p13) de novo
- 45,XX,rob(13;14) pat
- 45,XX,rob(13;14) pat
- 46,XY,t(5;8)(q31.3;p11) pat
- 46,XY,t(5;8)(q31.3;p11)
- 46,XY,t(1;7)(q31;q31) parents not tested
- 46,XY,t(6;7)(q25.3;q11.21) de novo*

**Patients referred with MRC/CA**

- 46,XX,inv(X)(p11.2p12.13) de novo
- 46,XX,inv(2)(p21.1;q13) de novo
- 46,XX,t(6;13)(q15;q22) mat

### Unbalanced rearrangements

**Patients referred with developmental delay only**

- 46,XX,del(17)(p11.2p11.2) de novo
- 46,Xdel(X)(q27.1q27.3) de novo
- 46,Xp+Y (present in an affected male sib)
- 46,XY,ins(14;?)q(21;?) mat
- 46,XX,del(10)(q11.21p11.23) de novo
- 46,XY,15p+ de novo
- mos46,XX/46,XX,−13,+rob(13;13) de novo
- 46,XY,del(17)(p11.2p11.2) de novo
- 46,XX,1p+ parents not tested
- 46,XY,t(22)(p11q13) parents not tested
- 46,XY,del(15)(q11q12) de novo
- 46,XY,14p+ de novo
- 46,XX,del(6)(q11q14) (adopted)
- 46,XX,r(22)(p11q13) de novo
- 46,XY,del(17)(p11.2p11.2) de novo
- 46,XY,+bisat dic mar de novo
- 47,XY,+bisat mar de novo
- 46,XY,15q+ de novo*
- 46,XY,del(2)(p11p13), t(3;11)(p32;q13) de novo*
- 46,XX,8p+ de novo*
- 46,XY,7q+ mother normal, father not tested*
- mos47,XX,+mar1/47,XX,+mar2/48,XX,+mar1;mar2 de novo*

**Patients referred with MRC/CA**

- 46,XY,del(3)(q24q24) de novo
- 46,XX,del(15)(q12q15) de novo
- 46,XX,del(18)(p11.2) pat
- 46,XY,13q+ de novo
- 47,XX,+bisat dic mar de novo

### Sex chromosome aneuploidies

**Patients referred with developmental delay only**

- 47,XXY
- 48,XXYY
- 47,XY
- 47,XX
- mos46,XY/47,XXY
- 47,XXX
- 47,XXY
- mos46,XY/47,XXY
- mos46,XY/47,XXX

**Patient referred with MRC/CA**

mos45,X/46,XX

*Classified as having only developmental delay on the basis of the provisional diagnosis, but on review found to have significant physical abnormalities.*