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.¹ About one-third of heterozygous carriers are clinically affected.² A wide range of IQ is observed in both males and females who express the fragile site.¹ Some phenotypically normal males have transmitted the syndrome.³ 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.^{4 5}

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.

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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 REFERRED WITH DEVELOPMENTAL DELAY ONLY

Abnormalities other than the fragile X were found in 44 (4.9%) patients. The abnormalities included

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%.

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 aneuploidy (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

TABLE 1 *Cytogenetic results in patients examined for fragile X.*

Provisional diagnosis	Normal results	Abnormal results			Total investigations
		Fragile X	Other abnormalities	Total abnormalities	
Developmental delay	821	27 (3.0%)	44 (4.9%)	71 (8.0%)	892
MR/CA	107	4 (3.3%)	9 (7.5%)	13 (10.8%)	120
All referrals	928	31 (3.1%)	53 (5.2%)	84 (8.3%)	1012

TABLE 2 *Fragile X detection in males and females.*

		Male	Female	Total
Intellectually impaired subjects	Total investigations	516	486	1012
	Fragile X positive	23 (4.5%)	8 (1.6%)	31 (3.0%)
First degree relatives of index cases	Total investigations	15	41	56
	Fragile X positive	5 (33.3%)	18 (43.9%)	23 (41.1%)

dysmorphic or to have minor facial anomalies. This is consistent with the general observation that the fragile X syndrome is not associated with congenital abnormalities.

CYTOGENETIC RESULTS IN THE FOLLOW UP OF FAMILIES OF INDEX CASES

Follow up of families with the fragile X is important for the prevention of the disorder through prenatal diagnosis and selective termination of pregnancy. Our follow up is incomplete, but to date we have examined 56 first degree relatives including 21 obligate carrier mothers, six of whom expressed the fragile X; two mothers with fragile X positive daughters, one of whom expressed the fragile X; five affected male sibs; and 18 female sibs, 11 of whom expressed the fragile X (table 2).

Discussion

This experience in a diagnostic cytogenetics laboratory of a large paediatric hospital confirms that the frequency of chromosomal abnormality in patients with developmental delay/mental retardation but no physical abnormalities is sufficiently high to warrant referral of all such patients, male and female, for cytogenetic investigation. A range of abnormalities can be expected in this group of patients.

As the abnormalities detected include both fragile X and subtle chromosomal rearrangements it is necessary to use banded preparations, and to examine X chromosomes from enough cells to ensure the detection of all abnormalities. Fragile site Xq27 is generally seen in less than 50% of metaphases of affected males and may be visible in only a small percentage of cells of heterozygous females. We chose to examine 70 cells for the fragile site Xq27. This ensures the detection of a 4% frequency of expression with a probability of 0.95, and the detection of a 6% frequency of expression with a probability of 0.99.⁶ Equivocal results were resolved by examining 200 cells or by analysing a repeat specimen. As examination for fragile X is time consuming, clinicians need to be careful to provide a full provisional diagnosis to the laboratory to avoid unnecessary analysis. Our data confirm the observation that the fragile X syndrome is not associated with congenital abnormalities. A more detailed description of physical abnormalities, or a clear statement that none was present, could reduce the number of full fragile X examinations needed to be carried out by at least 10%.

The level of abnormalities other than fragile X was 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.⁷

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. Warburton⁸ 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.⁹

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.

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APPENDIX Abnormalities detected other than fragile X.

Apparently 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)(p11.2q13) mat
46,XY,inv(12)(q12q21) pat
46,XY,t(1;16)(q12;p13) de novo
45,XX,rob(13;14) pat
45,XY,rob(13;14) pat
46,XY,t(5;8)(q31.3;p11) parents not tested
46,XY,t(1;7)(q31;q31) parents not tested
46,XY,t(6;7)(q25.3;q11.21) de novo*

Patients referred with MR/CA

46,Xinv(X)(p11.2p22.13) de novo
46,XX,inv(2)t(2;16)(p21q35;q21;q23) 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;?)(q21;?) mat
46,XX,del(10)(q11.2p11.23) de novo
46,XY,15q+ de novo
mos46,XX/46,XX,-13,+rob(13;13) de novo
46,XY,del(17)(p11.2p11.2) de novo
46,XX,8p+ parents not tested
46,XY,r(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
47,XY,+bisat dic mar de novo
47,XY,+bisat mar de novo
46,XX,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 MR/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,XXX
48,XXYY
47,XXY
47,XXY
mos46,XY/47,XXY
47,XXX
47,XXY
47,XXY
mos46,XY/47,XXY
mos46,XY/47,XXY

Patient referred with MR/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.