Original articles

Fragile X syndrome is less common than previously estimated

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Abstract

In 1986, a population study of school children in the city of Coventry gave an overall prevalence in males and females for fragile X syndrome of 1/952. The 29 children diagnosed as having fragile X syndrome in this study have been re-evaluated with molecular diagnostic techniques. Eighteen of the original 29 children have been found not to have the expansion of the FMR1 gene associated with fragile X syndrome. Revised prevalence figures have been calculated giving rise to an overall prevalence figure of 1/2720 (range 1/2198-1/3089). If the four children lost to follow up are also assumed not to have the fragile X syndrome, the revised prevalence figure was 1/5714 (range 1/4762-1/6349). Clinical review of boys with severe mental retardation from this and a subsidiary study show that the clinical features of head circumference greater than the 50th centile, testicular volume greater than the 50th centile, and IQ between 35 and 70 remain helpful in distinguishing boys with fragile X syndrome from those who have non-specific mental retardation.

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In 1986, a population study was performed among school children in the city of Coventry in order to ascertain the frequency of mental retardation associated with the fragile X chromosome.¹ The prevalence of fragile X syndrome in the 11 to 16 year old age group (the age of the greatest ascertainment) was found to be 1 in 952 and the fragile X syndrome has therefore been considered to be a major cause of mental retardation.

In 1990, Sutherland and Baker² described the non-pathological fragile site at Xq27.2 (FRAXD). Webb and Bunday³ therefore re-analysed their data from the Coventry study, excluding those children thought to have FRAXD. The numbers of affected boys fell from 16 to 14 and of affected girls from 13 to 11. These changes made little difference to the prevalence figure which was re-estimated as 1 in 1039. Moreover, the number of educationally subnormal (ESN) children at schools for mild

retardation (ESN(M)) with fragile X syndrome still exceeded those at schools for severe mental retardation (ESN(S)) in both boys and girls (17 ESN(M) and eight ESN(S)). Fragile X syndrome was therefore still considered to be an important cause of mental retardation, especially in children with mild to moderate mental retardation. It was pointed out in this revised article that the number of children diagnosed with fragile X syndrome in the West Midlands as a whole in the years between 1980 and 1984 was considerably less than would be expected according to the estimated prevalence.

In 1991, the fragile X gene (FMR1)⁴⁻⁶ was cloned, and we therefore analysed once more the data from the original study of Coventry school children. Since previous investigations have suggested that most children with the fragile X syndrome have mild to moderate mental retardation, we also re-evaluated the level of learning difficulties in the children still thought to have the condition.

Previous studies have tried to elucidate clinical pointers helpful in suggesting the diagnosis of fragile X syndrome in a child with otherwise unexplained mental retardation.⁷⁻⁹ In 1985, Thake *et al*¹⁰ addressed this in a population study of 156 boys with severe mental retardation. This series included the boys from the ESN(S) schools from the 1986 Coventry study and additional boys from five other education authorities. A total of 14 out of the 156 boys were diagnosed as having fragile X syndrome with cytogenetic testing. The clinical features which helped to distinguish the 14 boys with fragile X syndrome from those without were evaluated. This population of boys has also been evaluated using molecular diagnostic techniques and the usefulness of these clinical indicators reassessed.

Methods

Attempts were made to recontact all of the children from the two previous studies^{1,10} and permission was sought for a repeat blood sample to be taken from each subject previously diagnosed as being affected by fragile X syndrome.

Where permission was obtained, blood was taken for repeat cytogenetic and molecular analysis. Cytogenetic studies were performed using standard techniques with excess thymidine.

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Figure 1 Diagram to show the number of children at ESN(S) and ESN(M) schools in the “Coventry study” who are still thought to have fragile X syndrome.

Molecular analysis was performed using Southern blots of genomic DNA digested with *EcoRI* and *EagI* and hybridisation with the DNA

probe StB12.3 and also hybridisation of *PstI* digests with the DNA probe Ox0.55.¹¹ Having excluded children without the FRAXA trinucleotide expansion, revised prevalence figures were calculated. Clinical features in the children with FRAXA were compared with the group of children with a diagnosis of idiopathic mental retardation.

In children where FRAXA was excluded, a possible explanation was that they had FRAXE or FRAXF.^{12,13} FRAXE and FRAXF testing was therefore performed using Southern blots of genomic DNA digested with *HindIII* and hybridisation with the DNA probes OxE20 for FRAXE and OxF14 for FRAXF.^{14,15}

The level of expression of the fragile site in cases subsequently found not to have FRAXA was, where possible, reviewed.

Table 1 Results of cytogenetic and molecular testing for the boys in the study

Patient	Cytogenetic result (1986) (%)	Result of cytogenetic review (%)	Result of molecular testing	Test for FRAXE/F
MS	24	—	FM	—
PB	32	—	FM	—
PS	16	—	FM	—
RL	16	23	FM	—
RW	12	17	FM	—
DC	10	0	N	Neg
AG	6	0	N	Neg
SG	10	0	N	Neg
GE	7	—	N	Neg
PBS	4	4	N	Neg
MB	3	0	N	Neg
MO	5	—	N	Neg
IM	3	—	N	Neg
SH	5	4(FRAXD)	N	Neg
JA	5	—	—	—
AW	6	5(FRAXD)	—	—

FM=full mutation. N=no mutation. —=not done.

Table 2 Results of cytogenetic and molecular testing for the girls in the study

Patient	Cytogenetic result (1986) (%)	Result of cytogenetic review (%)	Result of molecular testing	Test for FRAXE/F
TG	24	—	FM	—
SGR	22	—	FM	—
MO	9	0	N	Neg
MD	5	—	N	Neg
NG	5	0	N	Neg
CJ	5	4	N	Neg
CO	3	0	N	Neg
EC	5	—	N	Neg
JC	4	—	—	—
MJ	5	4(FRAXD)	N	Neg
JO	7	7(FRAXD)	N	Neg
JG	7	—	—	—
LA	6	—	—	—

FM=full mutation. N=no mutation. —=not done.

Table 3 Prevalence of fragile X syndrome in Coventry school children with unexplained mental retardation before and after cytogenetic and molecular review

	ESN(S)				ESN(M)			
	1986*	1991†	1994a‡	1994b§	1986*	1991†	1994a‡	1994b§
Boys	1/10	1/12	1/15	1/15	1/16	1/18	1/80	1/159
Girls	1/8	1/8	0	0	1/10	1/13	1/21	1/52

* Figures for original “Coventry study”.

† After exclusion of cases with FRAXD.

‡ After exclusion of cases with no molecular evidence of FRAXA.

§ Assuming that the 4 children lost to follow up were FRAXA negative.

Results (fig 1, tables 1 and 2)

It was possible to recontact and rebleed 14 of the 16 boys and 10 of the 13 girls diagnosed as having fragile X syndrome in the “Coventry study”. The cytogenetics of the two boys lost to follow up had been re-evaluated in 1991 and one of these was thought to have the common rather than the pathological fragile site. The three girls lost to follow up had all had their cytogenetics reviewed in 1991. None of these was reclassified as FRAXD, although the result was unclear in one.

Of the 14 boys reclassified as having the fragile X syndrome in 1991, molecular analysis confirmed the diagnosis in only five and was not done in one. Of the 11 girls considered to have the fragile X syndrome in 1991, three were lost to follow up and only two now have the pathognomonic full mutation (none had a premutation). Therefore revised prevalence figures were calculated. Table 3 shows the prevalence figures according to sex and degree of learning difficulties.

As explained in the original paper,¹ it was felt that the most complete ascertainment was for children in the 11 to 16 age group. For this

Table 4 Prevalence of fragile X syndrome in Coventry school children aged 11–16 years before and after cytogenetic and molecular review

	Prevalence	Range
1986*	1/952 (1.05/1000)	1/772–1/1078
1991†	1/1039 (0.96/1000)	1/840–1/1178
1994a‡	1/2720 (0.37/1000)	1/2198–1/3089
1994b§	1/5714 (0.18/1000)	1/4762–1/6349

* Figures for original “Coventry study”.
† After exclusion of cases with FRAXD.
‡ After exclusion of cases with no molecular evidence of FRAXA.
§ Assuming that the 4 children lost to follow up were FRAXA negative.
Prevalence ranges have been calculated taking into account the possible differences in prevalence in the children not studied because their parents refused permission.¹

reason, the prevalence figures for all school children regardless of educational ability was calculated for this age group, and found to be 1 in 2720 as compared to 1 in 952 in 1986 (table 4). If it is assumed that the three children in this age group who were lost to follow up and who were not thought to have the common fragile site did not have FRAXA, the revised prevalence figure was 1/5714.

The results of the original “Coventry study” suggested that fragile X syndrome commonly causes learning difficulties of only a mild to moderate degree, although it could also cause severe learning difficulties in both sexes. Clearly, exclusion of those children now thought to have FRAXD and those with no molecular evidence of FRAXA, especially all three of the girls at ESN(S) schools and eight of the boys at ESN(M) schools, has altered this. Table 5 shows the IQ levels for both boys and girls thought to have fragile X syndrome after the original study in 1986, and following the exclusion of children with FRAXD or no molecular evidence of FRAXA. As expected, the girls tended to have higher IQs than the boys. No children, girls or boys, remain in the most severe group with an IQ of less than 20 and only two children (both boys) remain who had an IQ of less than 35.

None of the children who were found not to have FRAXA was subsequently found to have FRAXE or FRAXF to explain the fragile site which had been observed cytogenetically. The most recent cytogenetic results of these non-FRAXA, E, or F cases showed a low level of expression in more than half (expression $\leq 5\%$ in 11/18 and 6–10% in 7/18).

Table 5 Distribution of IQ among Coventry school children before and after cytogenetic and molecular review

IQ	1986*		1994†	
	Boys n	Girls n	Boys n	Girls n
>70	3	4	0	2(0)‡
50–70	8	5	3(2)	3(2)
35–50	2	4	1	0
20–35	2	0	2	0
<20	1	0	0	0
Total	16	13	6(5)	5(2)

* Figures from original “Coventry study”.
† After exclusion of cases with FRAXD or no molecular evidence of FRAXA.
‡ Figures in brackets are assuming that the 4 cases lost to follow up were FRAXA negative.

Table 6 Prevalence of fragile X syndrome obtained from population based studies and cytogenetic testing

Country studied	Population size	No studied	Prevalence	Reference
United Kingdom	28 570	347	1.05/1000	1
Finland	12 882	111	0.6/1000	17
Australia	112 735	675	0.3/1000	18
Sweden	40 871	89	0.6/1000	16

CLINICAL FEATURES OF FRAGILE X SYNDROME
It was possible to rebleed 13 of the 14 boys thought to have fragile X syndrome from the study of Thake *et al*¹⁰; the final boy refused further venesection. Three boys were found not to have evidence of FRAXA on molecular testing leaving 11 of the original 14 boys still thought to have fragile X syndrome. The clinical features of these remaining 11 boys have been re-evaluated and compared with the 145 boys with idiopathic mental retardation. This analysis showed no change in the differences in clinical features of boys with fragile X syndrome compared with the boys with idiopathic mental retardation that were reported in the original study of Thake *et al*.¹⁰

Discussion
There have been a number of studies using cytogenetic data to estimate the prevalence of fragile X syndrome^{16–18} (table 6), and a prevalence of around 1/1000 was widely quoted and accepted before molecular studies were possible. It has, however, been suspected for some time that this figure is too high,^{18–23} or if it is not too high, then the condition is being under diagnosed.^{23,24} Jacobs *et al*,²¹ in their molecular study of children with mental impairment, estimated the population frequency of fragile X syndrome to be 1 in approximately 9000 and a minimum population prevalence of 1 in 4130 was estimated by Slaney *et al*.²³ Since the study by Webb *et al*¹ is thought to have achieved complete ascertainment, this provided a very good opportunity to try and establish more accurate prevalence figures for a total population of school children. It came as no great surprise that fragile X syndrome is less common than originally thought, although it is perhaps surprising quite how many children have been excluded from the original study.

There are several possible reasons for the false positive diagnoses in the original “Coventry study”. As has already been mentioned, four of the Coventry school children were excluded once the common, non-pathological fragile site was recognised. Of the children excluded after molecular investigation, many only had low expression of a fragile site on cytogenetic testing. In the original study children were classified as “positive” for the fragile X if the abnormal chromosome was present in 3% or more of their cells. The current criteria used by our cytogenetics laboratory is $\geq 5\%$ for a positive result. Hence, although the diagnosis of fragile X syndrome was legitimate at the time, the diagnosis in many of the children would not have been made using current diagnostic criteria.

Of the seven non-FRAXA, non-FRAXD children, who had cytogenetic expression between 6 and 10% when originally studied, four were not found to express a fragile site on retesting in 1994. The reason for this is not clear, but may be related to the different culture conditions now used. In the 1986 study, culture media without available folic acid were achieved by a combination of direct deficiency and the use of folate antagonists together with excess thymidine, whereas current practice is to use excess thymidine alone.

Another explanation is that the non-FRAXA, non-FRAXD patients have FRAXE or FRAXF. Testing for expansions at these sites did not, however, find this to be the case. Although previous reports have suggested that FRAXE is a cause of learning disability,^{25,26} studies in some families have not shown a correlation between the FRAXE site and mental retardation.^{25,27} Whatever the relationship between the FRAXE fragile site and mental retardation, our findings would support those of others^{22,28} that FRAXE is very rare in the mentally retarded population. Studies of families with the FRAXF fragile site have not found a correlation between FRAXF and mental retardation.^{13,29} Our findings suggest that FRAXF, like FRAXE, is rare in the mentally retarded population and provide no evidence to suggest that FRAXF is a significant cause of learning disability.

Three girls and one boy have been lost to follow up, but for the purposes of calculating the prevalence figures, it was initially assumed that these children do have FRAXA. The one boy who was lost to follow up was at an ESN(M) school and had cytogenetic expression of the fragile site of only 5%. The three girls were all at ESN(M) schools and had cytogenetic expression of the fragile site of 4, 6, and 7% respectively. The boy had an IQ in the 50 to 70 range and two of the girls had IQs greater than 70. The IQ of the third girl was not known. Since all these children had relatively low cytogenetic expression and relatively high IQs, it is possible that some or all of them do not have the fragile X syndrome. If all four of these children were to be excluded, this would give a lowest possible prevalence figure of 1/5714 school children aged 11 to 16 (range 1/4762–1/6349) (table 4). Since three of those lost to follow up were girls, the least accurate estimate of prevalence is in this group. The final numbers are small, but if the four children who did not have molecular testing are excluded, the number of females with FRAXA is approximately half that of males (as compared with similar numbers in the original study).

Evaluation of the Coventry school children with respect to IQ and learning ability originally suggested that fragile X syndrome was a significant cause of disability for girls at ESN(S) schools (1 in 8) and also boys at ESN(M) schools (1 in 16). It is within these two groups, however, that the most dramatic number of exclusions have occurred. There are now no girls who were at ESN(S) schools, and only 1/80 boys who were at ESN(M) schools who actually have FRAXA. It can be seen from

table 5 using figures from the original "Coventry study" that all boys have been excluded from the two extremes of IQ, >70 and <20. Mild retardation (IQ 50–70) is still well represented (three out of six boys) but lower IQs (20–35 range) are better represented than previously (two out of six FRAXA cases). So, although fragile X syndrome is a significant cause of mild to moderate learning difficulties, this is not quite as marked as previously thought.

In 1985, Thake *et al*¹⁰ compiled a table summarising the usefulness of various clinical features as indicators of fragile X syndrome in boys at ESN(S) schools. The combination of apparently most useful features was IQ in the 35–70 range, head circumference greater than the 50th centile, and testicular volume in boys aged 10 or over above the 50th centile. This combination of features gave a 1 in 3.6 chance of picking a child with fragile X syndrome (compared with 1 in 9 for the group as a whole). Conversely, 1 in 5 boys in the fragile X syndrome group would have been missed using this combination of features. Re-evaluation of these figures after cytogenetic and molecular review showed that although this combination of features still helped as a clinical pointer for picking out children with fragile X syndrome from a group of children with idiopathic mental retardation at ESN(S) schools, a significant number would be missed (1 in 4) using these criteria. Since there are no clear cut clinical criteria to differentiate boys with fragile X syndrome from those who have non-specific mental retardation, testing for the fragile X syndrome is still recommended, but the above clinical features increase the chance of a positive result while at the same time excluding a number of boys with non-specific mental retardation.

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