Subtle chromosomal rearrangements in children with unexplained mental retardation

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Summary

Background No explanation for moderate to severe mental retardation is apparent in about 40% of cases. Although small chromosomal rearrangements may account for some undiagnosed cases, a lack of genome-wide screening methods has made it impossible to ascertain the frequency of such abnormalities.

Methods A fluorescence in-situ hybridisation (FISH) test was used to examine the integrity of chromosome ends in 284 children with unexplained moderate to severe retardation, and in 182 children with unexplained mild retardation. 75 normal men were also tested. When a chromosomal rearrangement was found, its size was estimated, and members of the child's family were investigated.

Findings Subtle chromosomal abnormalities occurred with a frequency of 7·4% in the children with moderate to severe mental retardation, and of 0·5% in the children with mild retardation. The abnormalities had an estimated population prevalence of 2·1 per 10 000, and were familial in almost half of cases.

Interpretation Once recognisable syndromes have been excluded, abnormalities that include the ends of chromosomes are the commonest cause of mental retardation in children with undiagnosed moderate to severe mental retardation. Owing to the high prevalence of familial cases, screening for subtle chromosomal rearrangements is warranted in children with unexplained moderate to severe mental retardation.

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Introduction

There are few clinically important disorders as common as mental retardation for which the pathogenesis is so poorly understood. Mental retardation affects about 3% of the population,[1,2] yet a diagnosis is obtained in only about a third of cases. Our poor understanding of its origins hampers the provision of effective treatment and preventive regimens, and remains a major challenge for medical practice.

The causes of mental retardation vary with the severity of the disorder: moderate to severe cases (defined as an IQ score <50) are much more likely to be due to a single pathological cause than are mild cases (IQ score 50–70), which are thought to be multifactorial in origin. Chromosomal and genetic disorders account for 30–40% of cases of moderate to severe mental retardation, environmental factors explain a further 10–30%, and the cause is unknown in about 40% of cases.[3–6] Genetic and environmental causes explain, in roughly equal proportion, about 30% of mild mental retardation; the cause is not known in the remaining 70% of cases.[7–10]

There is some evidence that small chromosomal rearrangements involving the terminal bands of chromosomes (subtelomeric regions) are an important unrecognised cause of mental retardation. Case reports have shown cytogenetically invisible subtelomeric rearrangements in mental retardation;[11–14] we carried out a pilot study of the frequency of such rearrangements in children with unexplained mental retardation. Once the low sensitivity and specificity of the test had been taken into account, we estimated that the prevalence of small subtelomeric rearrangements could be as high as 6%, but the 95% CI of our prevalence estimate was large (1–18%).[15]

The results of the pilot study strengthened our hypothesis that subtelomeric rearrangements are an important cause of mental retardation; however, the findings were inconclusive owing to the low sensitivity of the test and the small sample size. To address these issues, we first developed a new assay, based on fluorescence in-situ hybridisation (FISH), that had a sensitivity and specificity of almost 100%.[16,17] We now report a larger study to establish the prevalence of subtelomeric rearrangements in children with unexplained mental retardation, and to assess whether such chromosomal abnormalities have a role in the pathogenesis of this disorder. We also aimed to find out whether, when mental retardation is categorised by severity, the prevalence is the same across all groups, and whether rearrangements are more likely to arise de novo or to be inherited.

Methods

Sample selection

374 children (including young adults) with unexplained mental retardation were recruited from nine of 23 clinical genetics

	Number analysed	Abnormalities	Frequency (95% CI)
Moderate mental retardation	284	21	7·4 (4·4–10·4)
Mild mental retardation	182	1	0·5 (0–1·6)
Controls	75	0	0 (0–4·0)

Table 1: **Prevalence of subtle chromosomal abnormalities in children with mental retardation and in normal controls**

centres, and from two community learning disability teams in the UK. All cases with developmental delay or mental retardation, normal routine G-banded karyotype at a 550 band level, and no known cause were studied.

92 families in which at least one child had mild (n=78) or moderate (n=14) mental retardation were contacted through a population-based register of all children in the Cambridgeshire Health Authority who had special educational needs. For all such children whose parents agreed to participate in the study, a brief clinical examination was carried out, and a family history taken. Facial features (frontal and profile), and hands and feet were examined and photographed. Head circumference and height were noted. 5–10 mL blood was taken for chromosome studies.

The presence of dysmorphic features was assessed independently by two consultant clinical geneticists. Children were scored as having a minor congenital anomaly if the geneticists agreed on the presence of facial dysmorphism or minor congenital anomalies in the hand or foot. All parents were interviewed with a standard questionnaire about their child's development, and the resulting data, in addition to the information in the special-needs register, were used to assess the child's disability.

We chose as controls 25 healthy unrelated male volunteers and 50 randomly selected healthy fathers of children enrolled through the genetics clinics. A sample size was chosen such that a frequency of abnormalities less than 1% would be defined as unimportant. Ethical approval for our study was obtained from the appropriate ethics committees.

Procedures

Fixed chromosome suspensions were prepared either directly from the peripheral blood or from lymphoblastoid cell lines.[18] DNA was extracted by means of established protocols.[19] The multiprobe FISH protocol and the telomere-specific clones used in these studies have been described previously.[16,17] However, this study used clones from 12p, 6p, 9p, 20p, and 15q that are more telomeric than those reported previously, and clones which give unambiguous signals from their cognate chromosomes (unpublished results). The test examined 41 telomeres rather than 46, because we excluded the five acrocentric p arms that contain repetitive and ribosomal DNA only. All abnormalities detected in metaphases derived from cell lines were also analysed in metaphases prepared from peripheral blood samples.

The size of chromosomal rearrangements was estimated by a combination of microsatellite PCR analysis and FISH. Microsatellites were obtained either from genome databases (http://www.genome.wi.mit.edu) or from sequences from mapped clones.[20] Amplifications were carried out in 96-well polycarbonate plates, and analysed as previously described.[20] The patients' alleles were compared with those of each parent. When the PCR results were uninformative, clones that contained mapped microsatellite markers were identified by hybridisation of radioactively labelled PCR products to library filters. The chromosomal location of clones identified in this way was confirmed by FISH and by PCR. The extent of rearrangements was then investigated by FISH.

Statistical analysis

Comparisons between groups were with Fisher's exact probability calculation. CI were calculated on the assumption that the data were binomially distributed. Since two comparisons were made (between controls and mild mental retardation, and between controls and moderate to severe mental retardation) a Bonferroni correction was applied, and $p<0·025$ was taken as significant.

Results

Prevalence of chromosomal abnormalities

We analysed 284 children with moderate to severe retardation, and 182 children with mild retardation. The children enrolled from genetics clinics had moderate to severe mental retardation and minor congenital anomalies. 15% of the special educational needs group had moderate to severe mental retardation, and 21% had minor congenital anomalies. The group with mild mental retardation included 78 children from the special educational needs sample, and 58 children with mild mental retardation and no physical abnormalities. Re-examination of chromosomes with a conventional 550-band analysis revealed no abnormalities.

Familial rearrangements				De-novo rearrangements			
Karyotype	Size of monosomy	Size of trisomy	Parents	Karyotype	Size of monosomy	Size of trisomy	Parental origin of deletion
46,XY,der(12)t(6;12)(q27;p13.3)	15·5–22·0 cM	12·7–23·2 cM	Maternal balanced translocation	46,XY,del(1)(p36.3)	16·4–22·9 cM	. .	Maternal
46,XX,der(4)t(4;22)(p16;q13.3)	1·9–2·0 Mb	4·25–3·75 Mb	Paternal balanced translocation	46,XY,del(1)(p36.3)	16·4–22·9 cM	. .	Paternal
46,XX,der(9)t(9;13)(q34;p11.1)	0–6·3 cM	ND	Maternal balanced translocation	46,XY,del(1)(q44)	15·7–23·3 cM	. .	Maternal
46,XX,der(1)t(1;13)(q44;q34)	15·7–23·0 cM	0–15·8 cM	Maternal balanced translocation	46,XY,del(6)(p25)	6·4–8·6 cM	. .	Paternal
46;XY,der(4)t(4;11)(p16;p15.5)	>4·4 Mb	0·6–9·2 cM	Paternal balanced translocation	46,XY,del(9)(p24)	10·4–14·5 cM	. .	Paternal
46,XY,der(4)t(4;6)(q35;q27)	23·4–31·0 cM	42·1–54·2 cM	Paternal balanced translocation	46,XY,del(13)(q34)	30·0–30·6 cM	. .	Maternal
46,XX,der(1)t(1;19)(p36.3;q13.4)	0 cM†	0–5 cM	Paternal balanced translocation	46,XY,del(22)(q13·3)	0·1–1·0 Mb	. .	Paternal
46,XX,der(4)t(4;20)(q35;p13)	27·2–31·0 cM	>31 cM	Paternal balanced translocation	46,XY,del(22)(q13.3)	6–7 Mb	. .	Paternal
46,XY,der(8)t(8;20)(p23;p13)	0 cM†	25·0–30·6 cM	Maternal balanced translocation	46,XY,del(22)(q13.3)*	130 Kb	. .	Paternal
46,XX,der(7)t(2;7)(q37;q36)	8·5–12·0 cM	27·0–33·0 cM	Paternal balanced translocation	46,XX,der(9)t(9;16)(p24;q24)	0 cM†	>36 cM	Maternal
				46,XY,der(13)t(Y;13)(p11.3;q34)*	12·3–15·8 cM	3–5 Mb	Paternal
				46,XY,der(18)t(X;18)(q28;q23)	10·1–10·1 cM	0–16 Mb	Maternal

ND=not determined. *Already described.[15,27] †Rearrangement did not extend as far as the terminal marker of the human genetic map.
Mb=megabases; Kb=kilobases; cM=centimorgans

Table 2: **Extent and origin of chromosomal rearrangements**

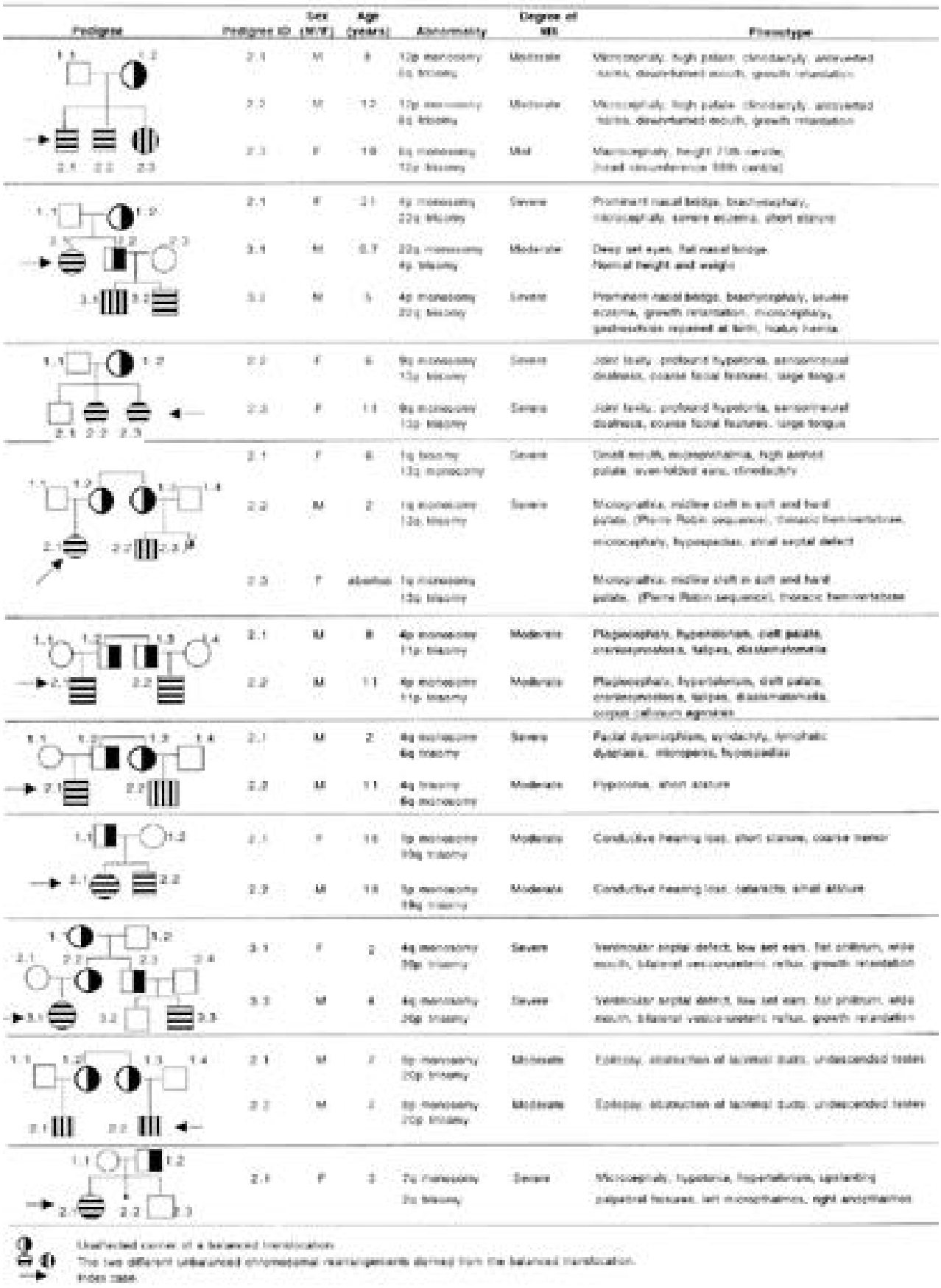

Figure 1: **Summary of familial rearrangements and associated phenotypes**

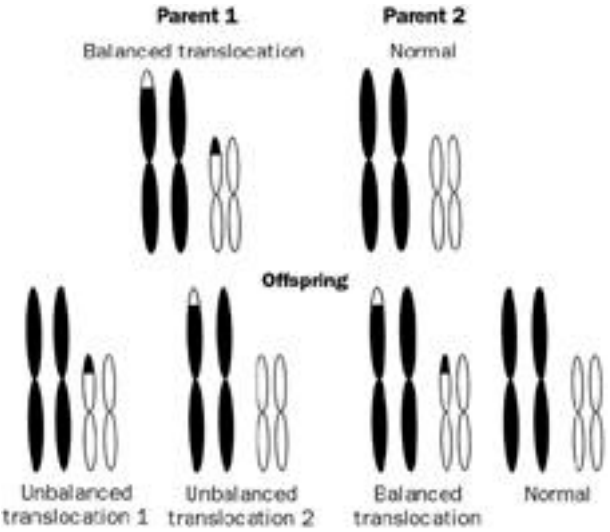

Figure 2: **Derivation of inherited chromosome abnormalities**

The prevalence of subtle chromosomal abnormalities in the group with moderate to severe mental retardation was 7·4% (table 1); this finding is consistent with the predictions of our pilot study.[15] The estimated frequency differed significantly from that in controls (p=0·0062, Fisher's exact test), and from that in children with mild mental retardation (p=0·0002, Fisher's exact test).

Characteristics of chromosomal abnormalities

We re-examined metaphase preparations from the affected individuals to see whether the chromosomal abnormalities could be detected cytogenetically. No abnormalities were found when the metaphases were examined without knowledge of the result of the FISH investigations at an 850-band level, but when known abnormalities were sought, they were found in four cases. These abnormalities were deletions involving 6p, 9p, and 13q, and a translocation between 1q and 13q (table 2). We have called the abnormalities detected using the multiprobe FISH assay "subtle chromosomal rearrangements" because routine cytogenic analysis had been reported normal on at least one occasion prior to our investigation.

We studied the parents of all 22 children with a chromosomal rearrangement (table 2). In 12 cases, the rearrangement occurred de novo and was not present in either parent. In nine of the remaining ten families, at least one sibling or other family member with mental retardation was available for testing. All affected family members had chromosomal abnormalities, whereas unaffected members were normal or had a balanced rearrangement (figure 1). Figure 2 shows the inheritance patterns found and how they arose. In families in which one parent has a balanced chromosomal rearrangement, there are two different unbalanced derivatives that the children may inherit (as well as a normal karyotype or the balanced rearrangement). This feature explains why different abnormal phenotypes segregate within the same family, as shown in figure 1.

	Cytogenetic abnormalities (n=5310)	Fragile X syndrome (n=394)
Autosomal		
Down's syndrome	17·4%	. .
Others	2·3%	. .
Sex chromosome	0·5%	. .
Fragile X (males)	. .	3·0%*

*Figures taken from three studies that used molecular diagnoses.[34–36]

Table 3: **Percentage frequency of cytogenetic abnormalities and fragile X syndrome in individuals with unexplained moderate to severe mental retardation[6,28–36]**

Assessment of size of rearrangements

We examined the extent of each monosomy and trisomy by a combination of PCR (looking for non-mendelian segregation of polymorphic microsatellite alleles) and FISH with probes that contain the mapped markers. All markers used have been mapped with a high degree of confidence,[20,21] but, with few exceptions, physical distances are not available. Therefore in most cases we report genetic disorders in centimorgans (cM; table 2).

Nine rearrangements affect chromosomal regions where monosomy is known to result in mental retardation. Mapping data confirmed that monosomies involved the critical regions of 1p- (two cases),[22] 4p- (Wolf-Hirschhorn syndrome; two cases),[23] 6p (one case),[24] 18q- (one case),[25] and 22q- (three cases).[26] In one case (monosomy 1p, trisomy 19q), the monosomy did not extend into the critical region for mental retardation. Difficulty in recognising the phenotype in the 4p- cases probably arose from the concurrent presence of trisomies (figure 1).

Discussion

Our results show that subtle chromosomal rearrangements are common in children with mental retardation, that the rearrangements cause the children's disabilities, and that half of the rearrangements are familial. These findings are important for advancing our understanding of the pathogenesis of mental retardation, and for the clinical management of affected children.

The frequency of subtle chromosomal abnormalities among children referred for investigation of unexplained moderate to severe mental retardation was 7·4%. In this group, the FISH assay detected more than twice as many cases as either cytogenetic or fragile X testing. When the cause of the disorder is not clinically evident, cytogenetic analysis detects abnormalities in about 2% of cases,[27] and cytogenetic surveys of cases with an IQ of less than 50 show that, once Down's syndrome is excluded, 2·8% of cases have a chromosomal rearrangement (table 3). About 3% of children are diagnosed as having fragile X syndrome by molecular methods (table 3). All other investigations have much lower detection rates than these methods. We suggest that subtle chromosomal rearrangements are the second most common cause of moderate to severe mental retardation after Down's syndrome.

Our estimate of the frequency of subtle chromosomal abnormalities in mild mental retardation (0·5%) is significantly lower than that in moderate to severe cases; however, mild mental retardation is so common that the number of cases with chromosomal abnormalities is large. If the population prevalence of unexplained moderate to severe mental retardation is 1·4 per 1000, and that of unexplained mild MR is 2·1%, the prevalence of subtle chromosomal rearrangements is 2·1 per 10 000.

Several factors may have biased our prevalence estimates. First, only 20% of patients with moderate to severe mental retardation were selected from a

population-based register (in Cambridgeshire) which may not have been typical of the whole UK. A further potential source of bias is the presence in our study of chromosomal abnormalities with recognised clinical phenotypes (4p-, 1p-, 9p-, 22q-, and 13q- deletions). However, the clinical phenotypes of these cases were judged to be atypical, so a more thorough initial clinical examination would not have led to their exclusion from our study. Finally, we have not excluded the presence of one form of subtle rearrangement involving chromosome ends, a small tandem duplication of DNA within the subtelomeric region, which may have been missed in our survey.

Our data support the view that the rearrangements are the cause of the children's disabilities. First, the prevalence of small chromosomal abnormalities in the group enrolled through the genetics clinics was significantly larger than that in the control group. However, the upper limit of the 95% CI is large (4%) for the control group (table 1), so although the comparison is consistent with a causal role for the deletions, it does not prove that one exists. Evidence for the biological effects of the rearrangements must also be considered.

An important observation is that unbalanced derivatives segregate with mental retardation, and, where there is more than one affected offspring, concordant phenotypes indicate the same unbalanced rearrangement, and discordant phenotypes indicate the presence of both rearrangements (figure 1). Furthermore, when family information cannot be used to show pathogenicity, mapping data show that the rearrangements either include regions previously found to be deleted in known mental retardation syndromes (eight cases), or are so large that they are almost certain to have an associated phenotype (four cases).

About half of the cases we examined were familial—ie, one of the parents was found to be carrying a balanced chromosomal rearrangement. The high rate of inherited anomalies has two important implications. First, because of the divergence of phenotypes due to the presence of contrasting chromosomal rearrangements (figure 2), we were able to establish a genetic diagnosis where one had not been previously considered. For instance, a cousin of one child with a small unbalanced translocation (fourth family in figure 1) had been diagnosed as having anoxic brain damage; in fact the cousin had the contrasting unbalanced chromosomal rearrangement. We found that a diagnosis can have unexpected ramifications for second-degree and third-degree relatives.

Second, the high frequency of familial translocations alters the balance in favour of screening of all children with moderate to severe unexplained mental retardation for subtle chromosomal rearrangements. The 21 positive results in the sample of 284 children with moderate to severe mental retardation resulted in informative tests being carried out in a further 49 family members (excluding the ten index cases). In familial cases, all results are informative, so the ratio of informative tests to the total screened is 25 per 100 (70 of 284), rather than seven per 100 if all cases had been sporadic. In other words, the cost per informative test is reduced three-fold, from US$5700 (assuming a cost of $400 per test) to $1600. We think that screening with subtelomeric probes would be a cost-effective way of making diagnoses in children with unexplained moderate to severe mental retardation.

Contributors

The project was conceived by Jonathan Flint. Samantha Knight coordinated the collection of samples, and analysed and interpreted molecular and FISH data; Patrick Bolton recruited cases in Cambridgeshire; Robin Winter and Tessa Homfray reviewed clinical data; Regina Regan, Alison Nicod, and Sharon Horsley contributed to the FISH data, and were responsible for the culture and harvest of metaphases; Lyndal Kearney supervised FISH analysis and cytogenetic interpretation of abnormal results; and Jonathan Flint and Samantha Knight wrote the first and final drafts of the paper. All investigators contributed to the writing of the paper.

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