ELECTRONIC LETTER

Subtelomeric rearrangements detected by FISH in three of 33 families with idiopathic mental retardation and minor physical anomalies

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Mental retardation (MR), defined as an intelligence quotient (IQ) of less than 70, affects 2-3% of the population and its aetiology and pathogenesis are still poorly understood. The aetiology can be established in only ~64% of cases with moderate to profound MR and in ~24% of cases with mild MR.[1] Available data indicate that chromosome aberrations are found in 4-28% of affected subjects. However, the yield of these abnormalities is increased when the severity of mental retardation and the presence of congenital anomalies are taken into account.

In the past decade, molecular-cytogenetic methods have documented a number of submicroscopic chromosomal rearrangements involving telomeric regions of chromosomes. They have been implicated in α thalassaemia with MR,[2–4] Wolf-Hirschhorn syndrome,[4–8] cri du chat syndrome,[4 9 10] and Miller-Dieker syndrome.[4 11] Their presence has also been reported in patients with 18p–, 18q–, 22q–, and 1p– deletion syndromes.[12–25] These observations suggest that the telomeric regions of chromosomes might be more prone to cryptic rearrangements and thus might be responsible for mental retardation. As telomeric regions of chromosomes have the highest gene concentration in the human genome, rearrangements involving these regions may have severe phenotypic consequences. Moreover, the molecular structure of telomeric regions and high frequency of recombination are predisposing factors to the occurrence of such rearrangements.[26 27]

At present, there is still no single, useful cytogenetic method for screening the entire genome, regardless of the size of suspected chromosomal abnormality. Classical cytogenetic analysis, even with the use of high resolution banding, enables the detection of abnormalities >3-10 Mb in size. Thus, the resolution of the method is not sensitive enough to identify subtle submicroscopic rearrangements. They are not detected by G banding not only because of their small size but also because of their localisation at the terminal light G bands, which are similar for most chromosomes and less readily distinguishable. In the first studies of telomeric regions in patients with mental retardation, Flint *et al*[28] used highly polymorphic markers to search for cryptic rearrangements. However, DNA polymorphism analysis requires DNA samples from both patient and parents and its use is limited by the informativeness of DNA markers. This analysis enables detection of deletion, duplication, and uniparental disomy cases but does not distinguish a normal person from a carrier of a balanced translocation.[28 29] Recently, primers used in microsatellite analysis were labelled with fluorochromes, which allowed the detection of the PCR amplification products on an automatic sequencer.[30] This innovation enabled the automated fluorescence analysis of subtelomeric regions.

Another strategy applied FISH with probes specific for subtelomeric regions of 22 autosomes and sex chromosomes.[31–34] The method was adapted for simultaneous analysis of the subtelomeric regions of every chromosome in one hybridisation test. The pair of probes for each chromosome was labelled with dual colours, allowing distinction between the telomeres of the p and q arms. This method, in contrast to the first one,

Key points

- Recently, it has been shown that subtelomeric chromosome rearrangements may be a common cause of idiopathic mental retardation (MR). We report the results of screening studies for subtelomeric aberrations in 33 families with cases of unexplained MR and multiple anomalies.

- There were 21 cases with moderate or severe MR and 11 with mild MR. Additionally, a healthy father of three children who had died and who were affected with severe MR was also included. Apart from MR, the main inclusion criteria for subtelomeric FISH were clinical features suggestive of a chromosomal aberration.

- Positive family history of MR was shown in 17 out of 33 examined cases. Fluorescence in situ hybridisation with a set of 41 subtelomeric probes was applied. Subtelomeric aberrations were found in 9% of cases (3/33). Two of three rearrangements were unbalanced and found in children with severe MR and physical anomalies. The third abnormality, a balanced reciprocal translocation, was present in a father of three dead children with severe MR. No abnormalities were found in patients showing mild MR. All identified aberrations were paternal in origin. Verification of the karyotype at a higher banding resolution level showed that two of them were half cryptic abnormalities. In the third case, both products of the identified translocation could be seen.

- Our results confirm previous findings that in order to exclude cytogenetically visible abnormalities in patients with idiopathic MR, high resolution analysis should be performed before subtelomeric FISH screening. They also indicate the important role of subtelomeric rearrangements in the aetiology of MR and emphasise the preferential occurrence of these abnormalities in patients with severe MR and positive family history of MR.

enables detection of both unbalanced and balanced rearrangements. Other methods enabling screening of the whole genome for chromosome aberrations, such as multipaint FISH (M-FISH) or CGH techniques, are not useful in detecting cryptic abnormalities because of their relatively low resolution, particularly limited for subtelomeric regions.[35–38] Recently, two new strategies, telomere DNA chips and a 12 colour multiplex FISH telomere assay (M-TEL), were applied.[38 39] It was also shown that some modification of CGH by the use of a restrictive fluorescence ratio threshold for background correction and normalisation enabled detection of cryptic subtelomeric translocations.[40] These methods are powerful diagnostic tools for subtelomeric rearrangement screening, but currently their utility is limited because they are not available in most diagnostic laboratories.

Recent reports indicate that subtelomeric aberrations occur with a frequency of 2-23% in patients with unexplained mental retardation.[28 30 41–47] These aberrations were mostly found in subjects with moderate or severe MR. Almost half of the reported aberrations were familial. The population prevalence of subtelomeric rearrangements was estimated as 2.1 per 10 000. However, further studies are needed for both assessing their role and establishing selection criteria for more cost effective testing of subtelomeric chromosome regions.

We have studied 33 families using FISH with a set of subtelomeric probes. Our study population included 21 patients with moderate to severe (IQ<49) MR and 11 with mild (IQ>50) MR and one healthy father of three children with severe MR who are now dead. Three subtelomeric aberrations were found. All three cases were familial and paternal in origin.

PATIENTS AND CLINICAL SELECTION CRITERIA

Thirty-three families with unexplained mental retardation were included. There were 21 cases with moderate to severe MR and 11 with mild MR. Additionally, a healthy father of three children with severe MR who had died was also included (he was the only person available for subtelomere testing in a family with many affected subjects). Patients were referred by a paediatrician, neurologist, or geneticist after detailed clinical and laboratory examination. Final qualification for subtelomere testing was determined by a clinical geneticist. The following inclusion criteria were used: unknown aetiology of MR, facial dysmorphism suggestive of a chromosomal aberration, coexistence of congenital malformations, abnormal prenatal and/or postnatal growth pattern, and a positive family history of mental retardation. Normal G banded karyotype at the 450-550 band level, normal results of the basic metabolic tests of urine, and exclusion of mutation within *FMR1* (fragile X syndrome) were prerequisites for inclusion in the study. In 17 out of 33 families studied, other mentally handicapped relatives or sibs were found. One or more miscarriages were reported in five families.

Case 1

The proband is a 5 year old boy (fig 1, IV.1), the first child of healthy and non-consanguineous parents (fig 1, III.1, III.2). The pregnancy was complicated by viral infection in the first trimester of pregnancy. He was born by normal delivery at 38 weeks of gestation to a 33 year old mother and a 33 year old father. His birth weight was 3250 g (50th centile), length 52 cm (95th centile), and head circumference 33 cm (10th centile). Apgar scores at one and five minutes of age were both 7. He required oxygen therapy because of mild respiratory insufficiency on the second day of life. General muscular hypotonia, microcephaly, and mild dysmorphic features (bilateral transverse palmar crease, high arched palate) were noted at birth. On further evaluation at 2 years, psychomotor development was significantly delayed and the following dysmorphic facial features were noted: microcephaly, high forehead, flat occiput, hypertelorism, epicanthic folds, short nose, bulbous tip of the nose, flat philtrum, open mouth, and downturned corners of the mouth. Camptodactyly of the fifth finger, abundant subcutaneous tissue on the dorsum of the hands, abnormal dermatoglyphics, and talipes valgus with absence of the nails on the fifth toe were also found. Psychological evaluation at 2 years 6 months confirmed severe motor and speech delay.

Pedigree analysis showed a positive history for mental retardation. The father's 40 year old sister (fig 1, III.3) was also severely mentally retarded. The phenotypic similarity between the proband and his aunt was visible by analysing their photographs from infancy and early childhood. She had short stature, microcephaly, facial dysmorphic features (hypertelorism, short, bulbous tip of the nose, short and flat philtrum), scoliosis, and talipes valgus. The second sister of the proband's father (fig 1, III.4) died on the fifth day of life. Congenital cardiac defect was suspected but not confirmed by clinical evaluation at necropsy.

Case 2

The proband is a 4 year old boy (fig 1, III.2), the second child of healthy and non-consanguineous parents (fig 1, II.1, II.2). He was referred for genetic evaluation because of dysmorphic features and psychomotor delay. He was born at 40 weeks of gestation to a 29 year old mother and a 32 year old father after a pregnancy complicated by one episode of vaginal bleeding and a urinary tract infection. His birth weight was 3750 g (50-75th centile), length 61 cm (90th centile), and head circumference 36 cm (75th centile). Apgar scores at one and five minutes of age were both 9. General muscular hypotonia and severe psychomotor delay were manifested during infancy and early childhood. Seizures were observed at 1 year 6 months and cerebral palsy was suspected. He did not speak until he was 3 and his psychomotor development was delayed. At the age of 3 years he manifested severe motor and cognitive retardation, facial dysmorphic features (prominent forehead, downward slanting palpebral fissures, arched eyebrows, long eyelashes, short philtrum, thin upper lip, microstomia), abnormal pattern of palmar dermatoglyphics, and a few "café au lait" macules on the arm, pectus carinatum, and talipes equinovarus. A history of inguinal and umbilical hernias was noticed, retrospectively. Pedigree analysis showed three cases of unexplained deaths during infancy or childhood in the grandfather's family (fig 1, I.1, I.2, I.3); however, no congenital abnormalities were reported.

Case 3

The proband (fig 1, IV.10) was born at 40 weeks of gestation after an uncomplicated pregnancy to a non-consanguineous and healthy 22 year old mother and a 25 year old father (fig 1, III.8, III.9). His birth weight was 3100 g (25th centile) and length 48 cm (70th centile). Apgar scores at one and five minutes were 6 and 7, respectively. On physical examination at 10 years of age, he showed the following features: profound psychomotor retardation, generalised hypotonia, microcephaly, hypospadias and bilateral cryptorchidism, 2/3 partial cutaneous syndactyly, abnormal sacrum, scoliosis, and dysmorphic features including hypertelorism, asymmetrical palpebral fissures, micrognathia, dysplastic ears, flat occiput, and high arched palate. He died at 16 years of age, before subtelomere testing could be performed. A routine G band chromosome analysis was normal. The second and third child (fig 1, IV.11, IV.12) died in the first days of life. The following congenital abnormalities and similar dysmorphic features in both of them were documented: prenatal growth retardation, microcephaly, cleft lip and palate, and talipes equinovarus. Polycystic kidneys and hypoplasia of the urethra and bladder were identified at the second child's necropsy (fig 1, IV.11). Two female sibs from the fourth and fifth pregnancy showed normal development without congenital defects (fig 1, IV.13, IV.14). Detailed analysis of the pedigree showed many mentally retarded subjects in the father's family. The clinical examination of one of them (fig 1, V.2) showed a peculiar phenotype similar to those of the affected family members. His karyotype was also normal. Two sisters of his mother had severe mental retardation and facial dysmorphism (fig 1, IV.4,
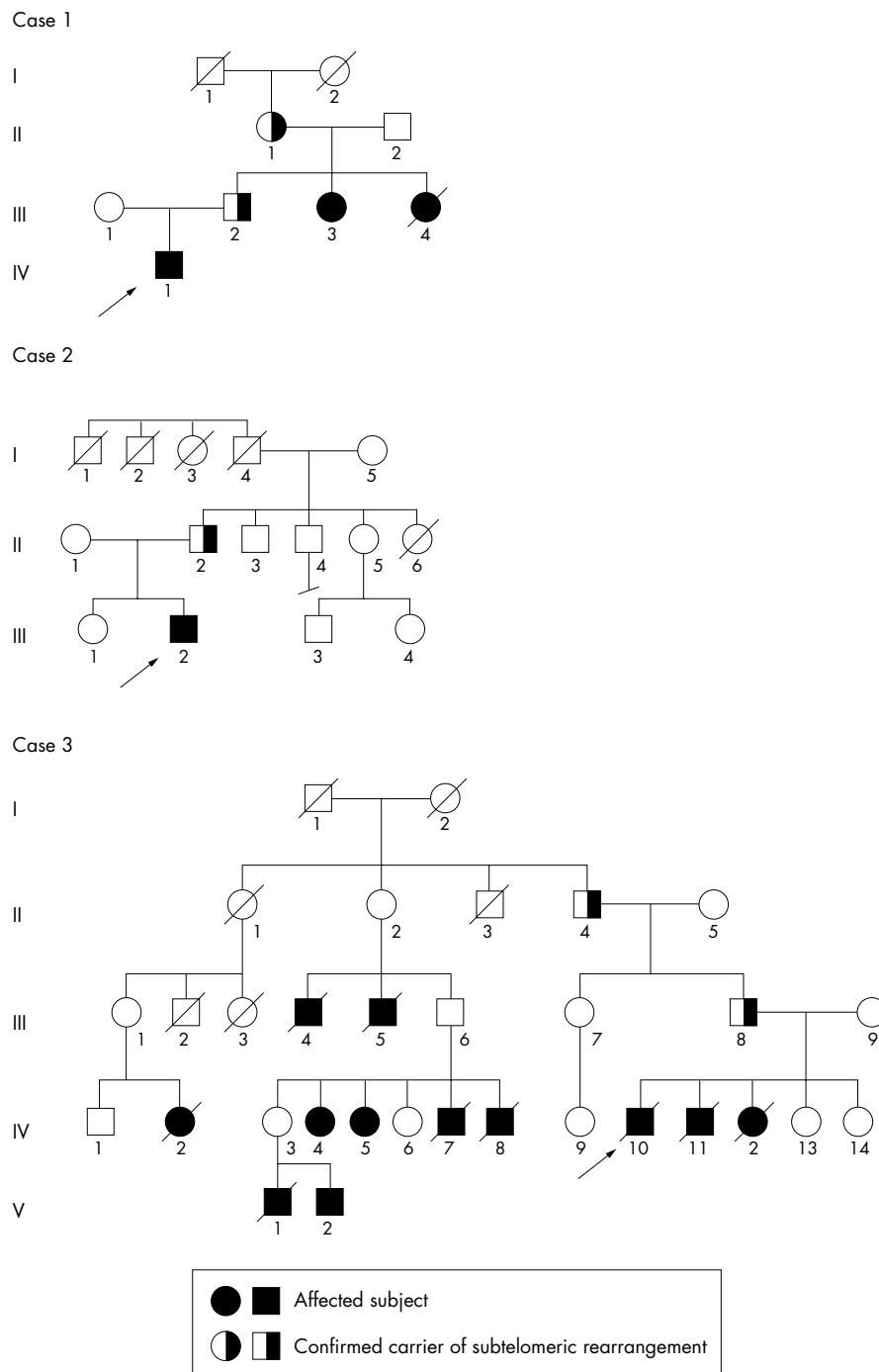
Figure 1 Pedigrees of three families with identified subtelomeric aberrations.

IV.5). They were not available for further clinical or cytogenetic evaluation. On the basis of the retrospective clinical studies, the presence of two different phenotypes in the affected subjects was noticed in this family.

METHODS
Cytogenetic studies
Chromosome slides were prepared from phytohaemagglutinin stimulated synchronised peripheral blood lymphocytes or fibroblasts using standard procedures. GTG banded chromosomes were analysed at the 450-550 band level. In the cases with identified subtelomeric rearrangements, the karyotype was verified at a higher resolution.

FISH studies
FISH with a set of 41 subtelomeric probes (Multiprobe Chromoprobe T System - Cytocell Ltd, UK) was applied for screening for chromosomal aberrations. Hybridisation was performed according to the procedure recommended by the manufacturer. The chromosome slides were prepared at a high temperature (60-80°C) and increased air humidity. One day old preparations were additionally treated for one to three hours with fresh fixative.

To reanalyse subtelomeric regions for which no fluorescence signals from Cytocell probes were seen or high background with faint fluorescence signals was present, a set of 41 PAC and BAC subtelomeric probes was applied (kindly provided by Dr J Flint).[34]

Table 1 Summary of FISH results of analysis of translocation breakpoints using unique and whole chromosome painting probes in carriers of balanced translocations

Translocation	Chromosome	Probe	Locus	Distance from the telomere (Mb)	Hybridisation signal on chromosome	
					der(4)	der(13)
t(4;13)(p16.3;q34)	4p	WHCR	D4S96	1.5	+	+
		815h5	D4S3032	4.2-5	+	+
		WCP4	Chromosome 4		+	+
	13q	785d7	D13S115	0.78	-	+
		929f12	D13S1315	1.2	-	+
		WCP13	Chromosome 13		+/-	+
					der(2)	der(7)
t(2;7)(q37;q36)	2q	770a05	D2S197	0.673	+	+
		854c09	D2S3381	2	+	+
		847e04	D2S2348	3.5-4.5	+	+
		WCP2	Chromosome 2		+	+/-
	7q	Oncor	D7S247	0.006	+	+
		79g3	VIPR2	2.69	+	+
		942g09	D7S637/D7S2447	3.5	+	+
		WCP 7	Chromosome 7		+	+
					der(7)	der(10)
t(7;10)(q36;q26)	7q	Oncor	D7S247	0.006	+	+
		965c12	D7S550	1.9	+	+
		942g09	D7S637/D7S2447	3.5	+	+
		WCP7	Chromosome 7		+	+
	10q	907b06	D10S1561	0.7-2.3	+	+
		WCP10	Chromosome 10		+/-	+

+/- faint hybridisation signal seen on the prometaphase chromosomes, + presence of hybridisation signals, - absence of hybridisation signals.

In three families in which subtelomeric rearrangements were recognised, other relatives were also examined using the probes specific for: Wolf-Hirschhorn critical region (WHCR - D4S96, Appligene Oncor) in case 1; YAC probe 770a05 (D2S1972 - 2q37.3, Centre d'Etude du Polymorphisme Human - CEPH, Paris) and cosmid probe 79g3 (VIPR2-7q36.3, Hospital for Sick Children, Toronto) in case 2; and locus D7S2447 (7q36.3), used as a reference probe together with the probe specific for Williams-Beuren critical region (WBCR - Appligene Oncor), in case 3. In the carriers of balanced subtelomeric translocations, the rearrangements were reviewed using whole chromosome painting probes (WCP Cambio, Appligene Oncor). These probes were used according to the manufacturers' instructions.

Additional more proximally located YAC probes were also used to characterise the positions of breakpoints on the derivative chromosomes (table 1).

YAC DNA obtained from MPI (Max-Planck-Institute, Berlin, Germany) was reamplified by DOP-PCR as recommended by the supplier. YACs were isolated from yeast strains and human sequences were amplified by *Alu* PCR reaction using the procedure described by Dr M Rocchi (www.biologia.uniba.it/rmc). Cosmid DNA was isolated using Qiaprep®Spin Miniprep Kit (Qiagen, Germany). BAC and PAC DNA was isolated using Nucleaband®PC 100 Kit (Macherey-Nagel, GmbH&Co KG, Germany). The PCR products and isolated cosmids, BACs, and PACs were analysed on 1% agarose gels to estimate their concentration. DNA was labelled with biotin-14-dATP using BioNickLabeling System (Gibco-BRL®, Life Technologies, USA). The labelling procedure was carried out according to the manufacturer's instructions. The probe DNA was dissolved in 50 μl of hybridisation solution: 50% formamide, 10% dextran sulphate, and 2 × SSC. FISH was performed according to a modified procedure of Pinkel *et al.*[48] Biotin labelled probes were visualised with fluorescein-avidin DCS and biotinylated antiavidin D antibodies (Vector Labs Inc, USA). Chromosomes were counterstained with 4,6-diamidino-2-phenylindole (DAPI) and propidium iodide (PI) diluted in Vectrashield antifade (Vector Labs Inc, USA).

The chromosome analysis was performed using an epifluorescence microscope Axioplan2 (Zeiss, Germany) equipped with appropriate filter combinations and with CCD KAF 1400 Photometrices camera. To document the microscopic images a computer system IP Lab Spectrum with Multiprobe extension software (Photometrics, USA) was used. For "home brew" and commercially available probes, 15-20 and 5-10 metaphases with signals on two homologous chromosomes were analysed, respectively.

RESULTS
Chromosome analysis at the 450-550 band level showed normal complement in all 33 patients. Further analysis using FISH with probes specific for subtelomeric regions showed chromosome rearrangements in three families (table 1).

Case 1
Hybridisation with subtelomeric probes specific for chromosomes 4 and 13 showed the presence of three fluorescent signals corresponding to 4p subtelomeric sequences, two on 4p and one on the tip of the q arm of a D group chromosome, and only one signal in the 13q subtelomeric region (fig 2A, B). Application of WCHR probe showed additional hybridisation signal on chromosome 13, in addition to two normal signals on chromosome 4p, indicating that this region was also involved in the translocation. In the proband's father, WHCR probe showed two signals, one in the normal chromosome 4p16.3 region and the other on derivative chromosome 13, showing that he was a carrier of a balanced translocation t(4;13)(p16.3;q34). The same translocation was found in the proband's grandmother. Thus, the proband's karyotype was designated 46,XY,der(13)t(4;13)(p16.3;q34)pat. The paternal sister carried the same unbalanced translocation as the proband. FISH analysis using more proximal YAC probes showed that the extents of trisomy 4p and monosomy 13q were at least 4.2 Mb and less than 0.78 Mb, respectively (table 1). The balanced translocation was re-evaluated at the 650
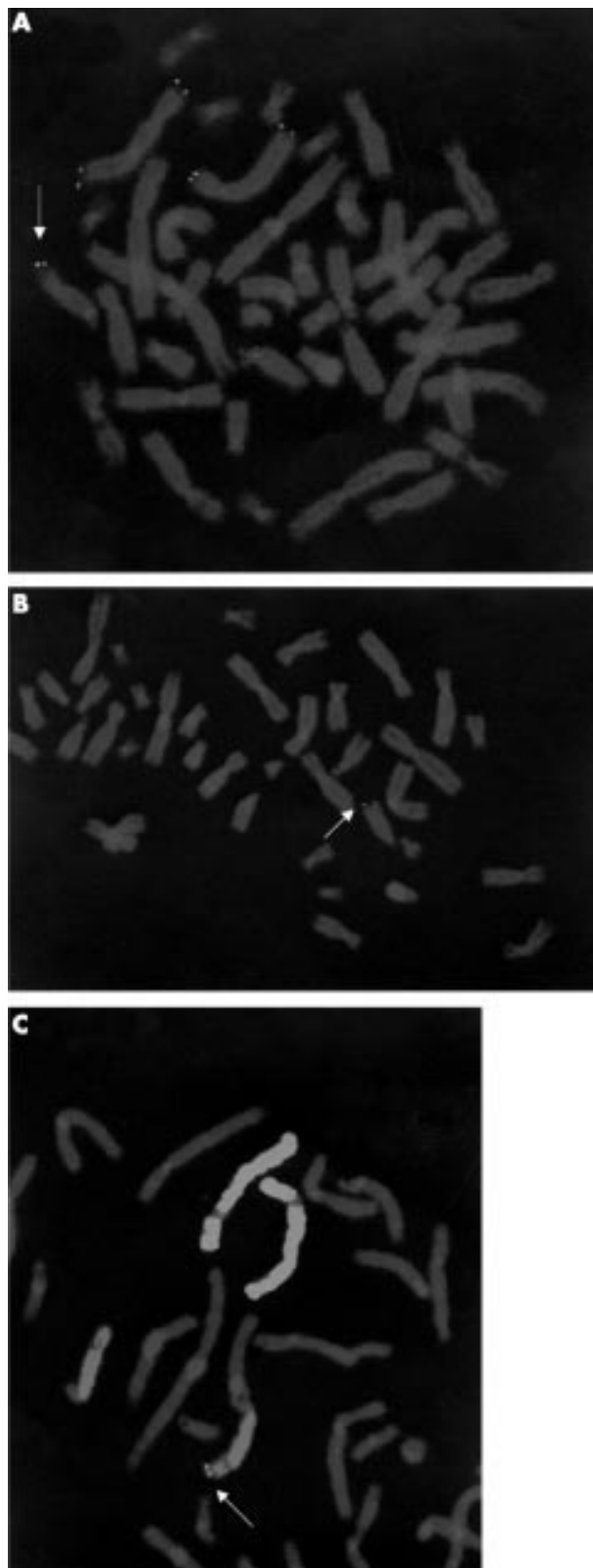
Figure 3 FISH results with subtelomeric probes specific for chromosome 7p and 7q subtelomeric regions in case 2 with the der(2)(2;7)(q37;q36). Two normal hybridisation signals of subtelomeric probes specific for 7p and 7q are seen on both chromosomes 7 and an additional signal of subtelomere 7q on chromosome 2q (arrow).

Figure 2 Results of FISH in case 1 (der(13)t(4;13)(p16.3;q34)). (A) Partial metaphase showing hybridisation signals on both normal chromosome subtelomeric regions 4p (green) and 4q (red) and an additional 4ptel signal on chromosome 13q (arrow). (B) Partial metaphase showing only one chromosome 13 with the signal of 13q subtelomeric probes (arrow). (C) Chromosome painting probes specific for chromosome 4 (green) and 13 (red) showed a small fragment of chromosome 13 painted with WCP4 (arrow).
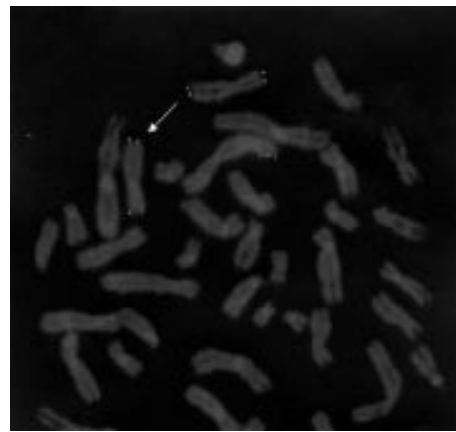
band level. The presence of extra small G negative material on one chromosome 13q and its loss on chromosome 4p was visible.

Case 2
The Multiprobe T FISH assay showed the absence of hybridisation signal on one of the chromosomes 2q and the presence of three signals specific for the 7q subtelomeric region. The additional signal was detected on the tip of the q arm of chromosome 2 (fig 3). The analysis of 2q and 7q subtelomeric regions in the proband's father showed that he was a carrier of a balanced reciprocal translocation t(2;7)(q37;q36) (fig 1, II.2). The proband's karyotype was designated 46,XY, der(2)t(2;7)(q37;q36)pat. In the proband's mother and his older sister, chromosomes 2 and 7 were normal (fig 1, II.1, III.1). Identified rearrangements involved regions of at least 3.5-4.5 Mb and 3.5 Mb from chromosomes 2 and 7, respectively (table 1). Re-examination of the paternal karyotype at the 650 band level showed a subtle deletion in the distal band of 7q, but no visible changes in the telomeric region of 2q could be detected.

Case 3
FISH analysis of subtelomeric regions showed that a father of three children who had died (fig 1, III.8) was a carrier of a reciprocal subtelomeric translocation t(7;10)(q36;q26) (fig 4). Unfortunately, chromosome preparations from affected children were not available. Subtelomeric regions of chromosomes 7 and 10 in two healthy daughters were normal (fig 1, IV.13, IV.14). Results of hybridisation with the cosmid probe mapping to locus *VIPR2* on chromosome 7q and with the WCP probes 7 and 10 suggested that the patient's father was also a carrier of the same reciprocal translocation (fig 1, II.4). FISH analysis using more proximal YAC probes showed that the extents of the aberration were at least 3.5 Mb for chromosome 7 and at least 0.7-2.3 Mb for chromosome 10 (table 1). Repeated G banding analysis at the 650 band level showed a deletion of a distal band in chromosome 7q36 but there was no visible change in banding pattern in chromosome 10q. Other family members would not consent to this examination.

DISCUSSION
An increasing number of identified cryptic aberrations localised in the telomeric regions has confirmed previous data that subtelomeric rearrangements are one of the important aetiological factors in mental retardation. Previous screening
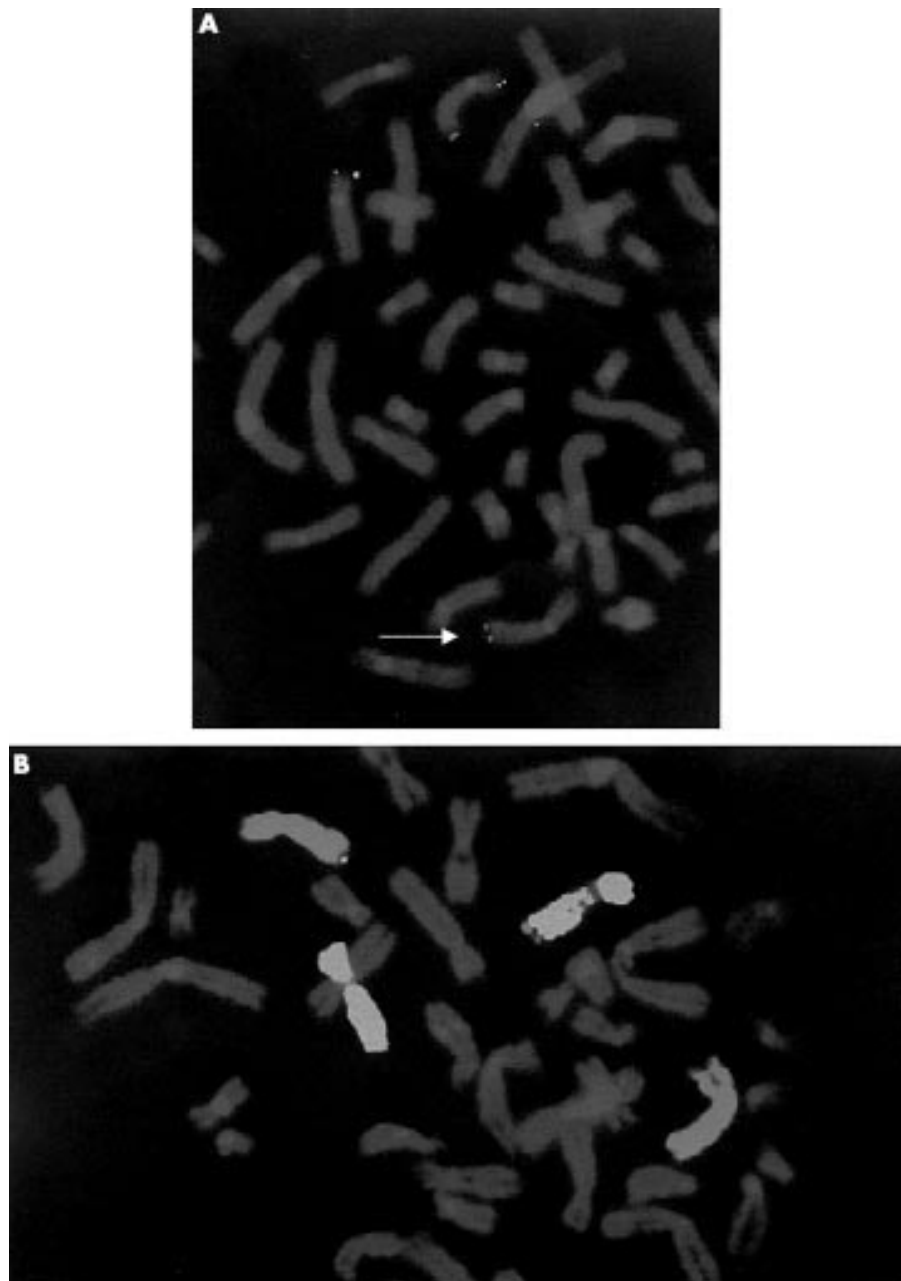
Figure 4 Results of FISH in case 3 (t(7;10)(q36;q26)). (A) Results of hybridisation with subtelomeric probes specific for chromosomes 10p and 10q. Hybridisation signals on two normal subtelomeric regions 10p (green) and only one normal signal on chromosome 10q can be seen (red). Signal specific for the subtelomeric region 10q can be seen on the tip of the q arm of one chromosome 7 (arrow). (B) Chromosome painting (WCP) with probes specific for chromosomes 7 and 10 showed that the fragments involved in the translocation could be detected on both chromosomes (chromosome 7 WCP10+, chromosome 10 WCP7+).

studies have shown that subtelomeric rearrangements occur with a frequency of 2-23% in subjects with unexplained MR.[28–30] [41–47] In the two largest screened cohorts of patients with moderate to severe MR, subtelomeric aberrations were found in 7.4% and in 10.2% of cases.[41] [46] In almost 50% of cases they were familial in origin. In our studies, the frequency of identified subtelomeric rearrangements in families with idiopathic MR was 9.1% (3/33), which is consistent with the prevalence reported previously.[41] [46] Our results confirmed the previous findings, showing the important role of familial subtelomeric abnormalities. All three identified subtelomeric cases had a positive family history of mental retardation and the rearrangements were paternal in origin.

It is possible that various patients' inclusion criteria might influence the frequency of identified rearrangements. How-ever, it is very difficult to establish precisely the clinical inclusion criteria for subtelomeric examination. The phenotype of affected children with subtelomeric aberrations can be very heterogeneous depending on the size and the origin of the chromosome rearrangements, as well as the number and function of genes involved. Detailed clinical studies in larger group of patients with already identified subtelomeric aberrations could be helpful in establishing selection criteria. Knight et al[41] stated that in children with telomeric defects a combination of facial dysmorphism, minor physical anomalies of the hands and feet, short stature, and microcephaly were often seen. However, a more important conclusion from their work is that screening for subtelomeric aberrations is especially warranted in subjects with moderate to severe rather than mild mental retardation. By detailed analysis of

clinical features observed in patients with these subtelomeric aberrations, De Vries et al[49] developed a checklist to facilitate patient preselection. The suggested checklist included: positive family history of mental retardation, prenatal and postnatal growth retardation, $\geqslant 2$ facial dysmorphic features, non-facial dysmorphic features, and/or congenital anomalies. Because of their significantly higher frequency in subtelomeric cases compared to controls, family history and prenatal growth retardation have been proposed by the authors as good indicators for screening for subtelomeric aberrations. The retrospective clinical analysis of our families with subtelomeric aberrations has shown the absence of prenatal growth retardation in two cases. In the third one (case 3), two of three dead children (fig 1, IV.11, IV.12) had prenatal growth retardation. In all affected subjects in our three families with identified subtelomeric aberrations, a combination of congenital anomalies (including hands and feet), $\geqslant 2$ facial dysmorphic features, hypotonia in the first year of life, and a positive family history of mental retardation was found. In addition, microcephaly in cases 1 and 3 and postnatal growth retardation in case 2 were noted. All affected subjects showed severe mental retardation. Thus, it seems evident from both our studies and from the previous observations that severe MR and clinical features suggesting chromosome aberration should be the main indication for subtelomeric FISH analysis.[41 46 49]

In our screening studies, the most commonly used assay, Chromoprobe Multiprobe T System, was applied. However, our experience shows that the successful analysis of all 41 subtelomeric regions in one hybridisation test is rarely possible. Fluorescence signals of some probes were faint or even not seen (most often for 5p, 9q, 12p, 12q, and 18p). For this reason the introduction of the supplementary set of subtelomeric clones could be necessary to complete the evaluation of the integrity of subtelomeric regions and to obtain unambiguous diagnostic results.

Identified subtelomeric rearrangements were reviewed at the 650 banding level. It was shown that translocations t(2;7) and t(7;10) (cases 2 and 3, respectively) were in fact half cryptic aberrations. Both derivative chromosomes of the translocation t(4;13) in case 1 could be identified at this resolution. These results confirm previous findings that high resolution chromosome analysis could identify some subtelomeric rearrangements overlooked in routine examination.[50] Thus, it should be performed before the subtelomere FISH screening. However, it is still unclear at which banding resolution level the patients with idiopathic MR should be karyotyped. Subjective and very time consuming analysis of all chromosomes at the 850 band resolution as well as similar banding patterns of telomeric regions should be considered when the optimal cost effective approach for diagnostic procedure is established. It should also be emphasised that unambiguous identification of subtle abnormality is easier in retrospective analysis when the aberrant chromosome is known.

The abnormal chromosomes were also examined by FISH with WCP probes (figs 2C, 4B). All except der(2) in translocation t(2;7) could be identified with these probes. These results show that some subtelomeric translocations could be successfully visualised using WCP probes. Multipaint FISH is not applied for subtelomeric screening studies because its resolution is not sufficient for analysis of these regions and chromosome painting cannot identify deletions.

We compared clinical findings in our patients with similar cases already published and in the Oxford Medical Databases. In case 1, with partial trisomy for the subtelomeric region of 4p and partial monosomy for the subtelomeric region of 13q, clinical features typical of both syndromes were observed in the child. Speech delay, muscular hypotonia, microcephaly, flat occiput, hypertelorism, high arched palate, bulbous nasal tip, camptodactyly of the fifth finger, hypoplastic nails, abnormal feet, and respiratory insufficiency were consistent with other previously reported cases with 4p duplication.[51–53] The 13q34 deletion was manifested in our proband by short stature and epicanthic folds.[54 55]

In case 2, a child had partial trisomy 7q36 and partial monosomy 2q37. Three other families with the same aberration have already been described.[41 56 57] A prominent forehead and arched eyebrows were the only common dysmorphic features observed in our child and these patients. Some features consistent with 2q37 deletion and 7q36 duplication described earlier were also found in our patient.[58–60]

In the third family, the clinical records of three mentally retarded, dead children of the t(7;10) carrier could be analysed only retrospectively. Some clinical features found in one of these children (mental retardation, microcephaly, hypertelorism, strabismus, and malformed ears) were also reported in the only published case with der(10)t(7;10)(q36;26).[61]

CONCLUSIONS

The results of our screening have shown that the prevalence of subtelomeric rearrangements was 9.1% in the families with idiopathic mental retardation and multiple anomalies. Our data confirm previous findings in the largest screened groups, showing that the frequency of subtelomeric rearrangements in patients with severe MR was 7.4% and 10.2%. They also underline that the occurrence of these aberrations is more common in people with severe MR and coming from families with more than one affected member. The results of our studies also show the importance of high resolution chromosome banding analysis before the subtelomeric FISH screening in patients with unexplained mental retardation. The important role of subtelomeric aberrations in the aetiology of mental retardation is commonly accepted, but further studies are needed to elucidate their prevalence and to establish more cost effective selection criteria for subtelomeric testing.

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