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ARTICLE

A novel automated strategy for screening cryptic telomeric rearrangements in children with idiopathic mental retardation

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Cryptic unbalanced subtelomeric rearrangements are known to cause a significant proportion of idiopathic mental retardation in childhood. Because of the limited sensitivity of routine analyses, the cytogenetic detection of such rearrangements requires molecular techniques, namely FISH and comparative genomic hybridisation (CGH). An alternative approach consists in using genetic markers to detect segmental aneusomy. Here, we describe a new strategy based upon automated fluorescent genotyping to search for non mendelian segregation of telomeric microsatellites. A total of 29 individuals belonging to 24 unrelated families were screened and three abnormal patterns of segregation were detected (two rearrangements and one parental disomy). This study gives strong support to the view that cryptic telomeric rearrangements significantly contribute to idiopathic mental retardation and demonstrates that fluorescent genotyping is a very sensitive and cost-effective method to detect deletions, duplications and uniparental disomies. *European Journal of Human Genetics* (2001) **9,** 319–327.

Keywords: mental retardation; telomere; chromosome rearrangement; automated genotyping; uniparental disomy

Introduction

Despite recent advances, the origin of largely 40% of moderate to severe cases of mental retardation (defined by an IQ score <50) remains unexplained.[1,2] Recognizing the cause of mental retardation has little therapeutic impact but helps in clarifying the recurrence risk and makes prenatal diagnosis and carrier testing feasible in affected families. Segmental aneusomy has recently been recognised as a significant cause of mental retardation.[3] However, standard cytogenetic analyses have only a 400–500 band resolution and may overlook subtle chromosome rearrangements in individuals with apparently normal karyotype. Indeed, cryptic subtelomeric rearrangements have eventually been observed in the alpha-thalassemia/mental retardation syndrome,[4,5] the Wolf-Hirschhorn syndrome (MIM 194190, deletion of terminal 4p),[6] the Miller-Dieker syndrome (MIM 247200, deletion of terminal 17p),[7] or the cri-du-chat syndrome (MIM-123450, deletion of terminal 5p).[8] Higher resolution analyses (850-band resolution) are feasible but they are time-consuming and rather more suited for the analysis of specific chromosomal regions.

For this reason, a molecular cytogenetic approach based on fluorescence *in-situ* hybridization with telomere-specific probes and a multiprobe coverslip has been developed,[9] and

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has led to the conclusion that subtle chromosomal abnormalities occur in 7.4% of severely retarded patients.[10] However, this method requires the preparation of high mitotic index chromosomes and remains difficult and expensive for routine diagnostic tests. Similarly, comparative genomic hybridisation (CGH) is a valuable technique but this approach is unable to detect subtle anomalies (below 5 Mb) and requires specific microscope and computer facilities.[11]

On the other hand, microsatellite markers are naturally occurring DNA polymorphisms that can be used to search for irregular allele inheritance and detection of deletions, duplications and uniparental disomies.[12] Recently, a pilot study based on conventional genotyping allowed diagnosis of two deletions of telomeric regions in a series of 27 children with idiopathic mental retardation.[13] We have developed a novel strategy based on automated fluorescent genotyping using a new set of telomere-specific markers. Here, we show that automated fluorescent genotyping can be successfully adapted to the detection of cryptic terminal unbalanced translocations and uniparental disomies in patients with idiopathic mental retardation. This approach will hopefully help to determine the actual frequency of these rearrangements in idiopathic mental retardation and may lead to the identification of unknown disease genes and imprinted regions.

Materials and methods

Patients

A total of 29 children (17 boys and 12 girls, belonging to 24 families) born to unrelated parents and presenting with moderate or severe idiopathic mental retardation (IQ below 50) and non-syndromic dysmorphic features were recruited from the Department of Genetics of the Hopital Necker. Inclusion criteria were the presence of at least one of the following: (1) seizures; (2) overgrowth or failure to thrive; (3) behavioural anomalies (hyperactivity, aggressiveness or automutilation; and (4) congenital anomalies. In addition, all patients had a normal karyotype using both RHG and GTG banding analysis at 500–550 band resolution level. Their clinical features are summarised in Table 1.

Microsatellite markers, PCR amplification and GeneScan analysis

The number, location and heterozygosity scores of the microsatellites are shown in Table 2 and the data were obtained from either the GENETHON database (http://www.genethon.fr/) or the GENOME DATABASE (http://www.gdb.org/). Primers labelled with a NED fluorochrome were ordered from PE Biosystem (Courtabeuf, France) and primers labelled with a 6-FAM or HEX fluorochrome were ordered from MWG BIOTECH (Courtabeuf, France). DNA was extracted from peripheral blood lymphocytes according to standard techniques. PCR amplification of genomic DNA from parents and children (100 ng) was separately performed in 96-well microtitre plates using a Perkin-Elmer 9700 thermocycler and the PE Biosystems AmpliTaq Gold™ according to manufacturer's instructions. PCR products were then pooled according to their size and labelling (see Results) and analysed on an automatic sequencer (ABI 377, PE Biosystem) according to the manufacturer's recommenda-

Table 1 Summary of rearrangements and associated phenotype

	Patient	*Anomaly*	*Degree of MR*	*Phenotype*
Family 1: t(6;10)(q27;q26.3)				
I	II.1	6q monosomy 10q trisomy	severe	Long and thin face, microstomia, dental anomalies, tall stature, cleft lip and palate, developmental delay, seizures, hyperactivity
II	II.3	6q trisomy 10q monosomy	severe	Microcephaly, strabism, proeminent nasal bridge, thin upper lip, microretrognatism, normal ears
1 2 3				
Family 3: del 10q26.3				
	II.1	10q monosomy	severe	Enophtalmia, long nose, full lips and everted lower lip, foot deformation, autism
I	II.2	10q monosomy	severe	Enophtalmia, long nose, full lips and everted lower lip, foot deformation, autism
II	II.3	unknown	mild	No dysmorphy, normal language
	II.4	not tested		Died at age 2 years
1 2 3 4				
Family 4: UPD of 17q25.3				
I	II.1	UPD of 17q25.3	severe	Macrostomia, thick upper lip, hypertelorism epicanthus, broad nasal bridge, aggressive behaviour
II				

◧, unaffected carrier of a balanced translocation; ▨, ▧, two different unbalanced chromosomal rearrangements derived from the balanced translocation; ▩, deceased child.

Table 2 Microsatellite markers used for telomere genotyping

Telomere	D Number	Genetic distance to telomere (cM)	Fluorophore	Size range	Heterozygosity
1p	D1S243	0	FAM	142–170	0.86
	D1S468	6.2	HEX	173–191	0.75
1q	D1S2682	0	NED	110–150	0.76
	D1S2836	2.6	FAM	268–281	0.79
2p	D2S2268	0	HEX	205–231	0.61
	D2S323	3.6	FAM	177–193	0.57
2q	D2S2338	0	FAM	155–173	0.55
	D2S140	4.5	NED	151–167	0.76
3p	D3S1270	0	NED	154–196	0.75
	D3S1307	1.4	FAM	237–251	0.80
3q	D3S3550	0	FAM	230–270	0.74
	D3S3707	2.2	HEX	262–278	0.65
4p	D4S3038	0	NED	195–240	0.78
	D4S2936	0	NED	170–184	0.83
4q	D4S2930	0	NED	216–236	0.80
	D4S426	1.1	HEX	177–191	0.56
5p	D5S678	0	FAM	252–264	0.71
	D5S392	0.6	HEX	83–117	0.87
5q	D5S2006	0	HEX	135–165	0.74
	D5S408	1.8	FAM	247–265	0.73
6p	D6S1600	0	FAM	165–210	0.77
	D6S344	1.4	HEX	139–159	0.72
6q	D6S281	0	HEX	125–180	0.68
	D6S1693	10.8	FAM	85–93	0.51
7p	D7S2477	0	FAM	132–180	0.66
	D7S2563	1.7	HEX	91–111	0.78
7q	D7S2423	0	NED	230–270	0.71
	D7S2465	1.9	HEX	158–181	0.83
8p	D8S504	0	FAM	193–203	0.71
	D8S264	0.7	FAM	121–145	0.83
8q	D8S1836	0	HEX	121–160	0.84
	D8S1751	1.4	NED	149–163	0.69
9p	D9S1779	0	NED	114–154	0.63
	D9S1858	0.1	HEX	124–144	0.58
9q	D9S1838	0	NED	155–175	0.83
	D9S158	3.5	FAM	213–233	0.71
10p	D10S249	0	NED	106–152	0.74
	D10S558	1.9	NED	192–212	0.80
10q	D10S1700	0	HEX	107–148	0.64
	D10S212	1	HEX	189–201	0.70
11p	D11S1363	0	FAM	240–260	0.59
	D11S4177	3.1	FAM	183–213	0.80
11q	D11S968	3.3	HEX	131–165	0.81
	D11S4125	3.3	FAM	231–253	0.81
12p	D12S352	0	HEX	145–170	0.72
	D12S341	0.6	FAM	114–130	0.79
12q	D12S357	0	HEX	260–315	0.84
	D12S1638	0	NED	120–130	0.68
13q	D13S293	0	NED	80–106	0.50
	D13S1295	4.6	HEX	102–120	0.74
14q	D14S1007	0	HEX	110–135	0.77
	D14S293	3.9	HEX	153–165	0.65
15q	D15S966	0	HEX	225–265	0.82
	D15S203	0.6	FAM	116–140	0.62
16p	D16S521	0	FAM	156–172	0.71
	D16S3024	5	HEX	208–248	0.86
16q	D16S3121	0	HEX	65–90	0.62
	D16S3407	0	FAM	195–205	0.77
17p	D17S1866	0	HEX	144–195	0.80
	D17S926	0.6	FAM	243–260	0.80
17q	D17S928	0	NED	76–96	0.75
	D17S1830	11	HEX	108–122	0.79
18p	D18S59	0.1	HEX	127–182	0.81
	D18S476	1.6	HEX	263–275	0.76
18q	D18S70	0	FAM	115–135	0.83
	D18S1141	1.9	NED	263–293	0.77
19p	D19S886	0	NED	105–165	0.63
	D19S883	5.5	HEX	158–170	0.72
	D19S878	6.5	FAM	208–230	0.83
19q	D19S890	0	HEX	190–210	0.68
	D19S218	2.5	FAM	240–256	0.60
20p	D20S864	0	FAM	215–235	0.52
	D20S103	0	FAM	92–106	0.70
20q	D20S173	0	HEX	135–195	0.66
	D20S171	2.1	FAM	123–149	0.73
21p	D21S1911	0.1	HEX	105–145	0.69
	D21S1904	0	HEX	152–170	0.52
21q	D21S1897	0	FAM	178–224	0.74
	D21S1903	0.7	FAM	243–265	0.83
22q	D22S1169	0	FAM	110–135	0.78
	D22S1161	2.2	HEX	235–245	0.68
Xp	DXYS233	0	HEX	260–300	0.81
	DXYS234	2	NED	234–254	0.72
Xq	DXS8087	0	FAM	270–300	0.69
	DXS1073	1.6	HEX	217–243	0.80

Genetic distance from centromere and heterozygosity score were obtained from the Genethon map (http://www.genethon.fr).

tions. An internal ROX-labelled size standard (400HD-ROX 1 fmol per lane; PE Biosystems) was added to each lane to ensure precise sizing of PCR products and minimise lane-to-lane variation. Gel lane tracking and sizing of the size standard peaks were checked manually for all lanes. GENESCAN™ and Genotyper™ softwares (Applied Biosystems) were used to size the PCR products and to analyse the data according to the manufacturer's instructions.

Chromosome and FISH studies

Metaphase spreads were prepared from phytohemaglutinin (PHA) stimulated blood lymphocyte cultures using standard procedures of hypotonic treatment and methanol/acetic acid fixation (3 : 1). RHG and GTG banding methods were performed according to standard protocols.[14]

Two sets of subtelomeric FISH probes were used for hybridisation. The first set of probes corresponds to the Chromoprobe Multiprobe T System commercially distributed by Cytocell and is composed of telomeric-specific cosmids and PAC clones previously tested on unrelated individuals to exclude polymorphisms.[9] The second set of probes is composed of 41 well characterised CEPH YACs specific of each subtelomeric region and located about 2–3 Mb away from the telomere.[15] They were kindly provided by Thomas Haaf. YAC DNA were isolated by pulse-field gel electrophor-

Table 3 Sets of PCR-amplified dinucleotide repeat microsatellite markers suitable for automated fluorescent genotyping

Locus	*Telomere*	*Labelling*	*Allele size range*
SET 1			
D1S243	1p	FAM	142–170
D1S2682	1q	NED	110–150
D2S2268	2p	HEX	205–231
D4S3038	4p	NED	195–240
D3S3550	3q	FAM	230–270
SET 2			
D2S2338	2q	FAM	155–173
D3S1270	3p	NED	154–196
D5S678	5p	NED	252–264
D5S2006	5q	HEX	135–165
D6S281	6q	HEX	203–219
SET 3			
D4S2930	4q	NED	216–236
D6S1600	6p	FAM	165–210
D7S2423	7q	HEX	230–270
D8S1836	8q	HEX	121–160
D9S1779	9p	NED	114–154
SET 4			
D7S2477	7p	FAM	132–180
D8S504	8p	FAM	193–203
D9S1838	9q	NED	155–175
D11S968	11q	HEX	131–165
D12S357	12q	HEX	260–315
SET 5			
D10S249	10p	NED	106–152
D10S1700	10q	FAM	107–148
D11S1363	11p	FAM	240–260
D12S352	12p	HEX	145–170
SET6			
D14S1007	14q	HEX	110–135
D15S966	15q	HEX	225–265
D16S521	16p	FAM	156–172
D16S3407	16q	FAM	195–205
D13S293	13q	NED	80–106
SET 7			
D17S1866	17p	FAM	144–195
D17S928	17q	NED	76–96
D18S59	18p	HEX	127–182
D18S70	18q	FAM	115–135
D19S886	19p	NED	105–165
SET 8			
D19S890	19q	HEX	190–210
D20S864	20p	FAM	215–235
D21S1911	21p	HEX	105–145
D22S1169	22q	FAM	67–85
DXYS233	Xp	HEX	260–300
SET 9			
D20S173	20q	HEX	135–195
D21S1897	21q	FAM	178–224
DXS8087	Xq	FAM	270–300

esis (PFGE) and amplified using the degenerate oligonucleotide-primed-polymerase chain reaction (DOP-PCR) procedure as previously described.[16] Probes were labelled with biotin-16-dUTP or digoxigenin-11-dUTP (Boehringer-Mannheim) using a commercially available random-priming kit (Gibco-BRL). Biotin-labelled probes were detected using Texas Red (TR) conjugated to avidin and digoxigenin-labelled probes were detected using fluorescein isothiocyanate (FITC) conjugated to anti-digoxigenin. Slides were counterstained with 4′, 6′-diamidino-2-phenylindole (DAPI). Image capture and analyses were performed using a Zeiss Axiophot epifluorescence microscope equipped with the appropriate filter combination for detecting TR, FITC and DAPI. The images were captured by a cooled CCD camera controlled using an image analysis system (Vysis). Ten hybridised metaphases were analysed for each probe.

Results

Analysis of chromosome ends using automated fluorescent genotyping

We first designed a set of 42 fluorescently labelled primer pairs that corresponded to the two most distal microsatellite markers (2 bp repeat) of the Genethon human linkage map.[17] Each pair was labelled with one of the three fluorescent dyes FAM, HEX or NED. For each individual, DNA was individually amplified for all loci. PCR products were then arranged into nine sets on the bases of allele sizes and colours of fluorescent labelling (Table 3) and electrophoresed in one lane on an automated sequencer (Figure 1A). Sizing of the fragments was then performed using the ABI GeneScan software (Figure 1B). For each marker, the genotype of the child was determined and compared to the parental genotypes to detect (i) missing alleles (deletion), (ii) the presence of a third allele (duplication) or (iii) the presence of one or two alleles from one parent with no contribution from the other parent (uniparental isodisomy or heterodisomy respectively). Whenever the child was heterozygous for two different alleles identical to that of his parents, the genotype was regarded as normal. In case of uninformative polymorphism, when the child was homozygous for one allele shared by both parents, the segregation of the closest microsatellite marker on the Genethon map was tested. A total of 29 children, belonging to 24 different families, and 48 parents were analysed and a total of 4478 genotypes were determined. We detected six cases of non-mendelian inheritance.

Identification of a t(6,10) translocation in family 1

The two affected children of family 1 presented abnormal genotypes for both chromosome 6q and 10q markers. The son inherited a single maternal allele at the D6S281 locus, suggesting therefore a telomeric deletion of chromosome 6q (Figure 2A). His sister had a normal genotype at this locus but inherited a single maternal allele at the D10S1700 locus (Table 4). To confirm these findings and estimate the extent of the imbalance, additional genotyping was performed with markers selected from the CEPH-GENETHON integraded

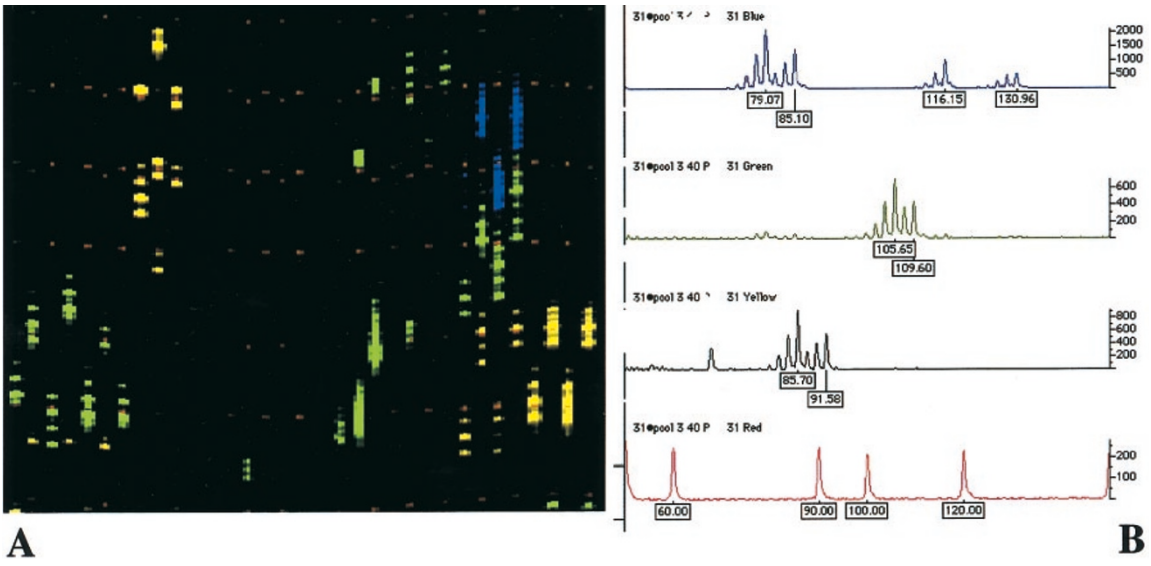

Figure 1 Extensive screening of children with idiopathic mental retardation. **A** Gel view from the ABI PRISM 377 DNA sequencer showing samples from 36 individuals (one per lane) and the ladder. The four loci are multiplexed on the bases of allele size and colours of fluorescent labels. The red bands are the internal size standards. **B** Genotyper electropherogram plots for one individual. The peaks shown in blue, green and black represent the amplified PCR products labelled with FAM, HEX and NED respectively.

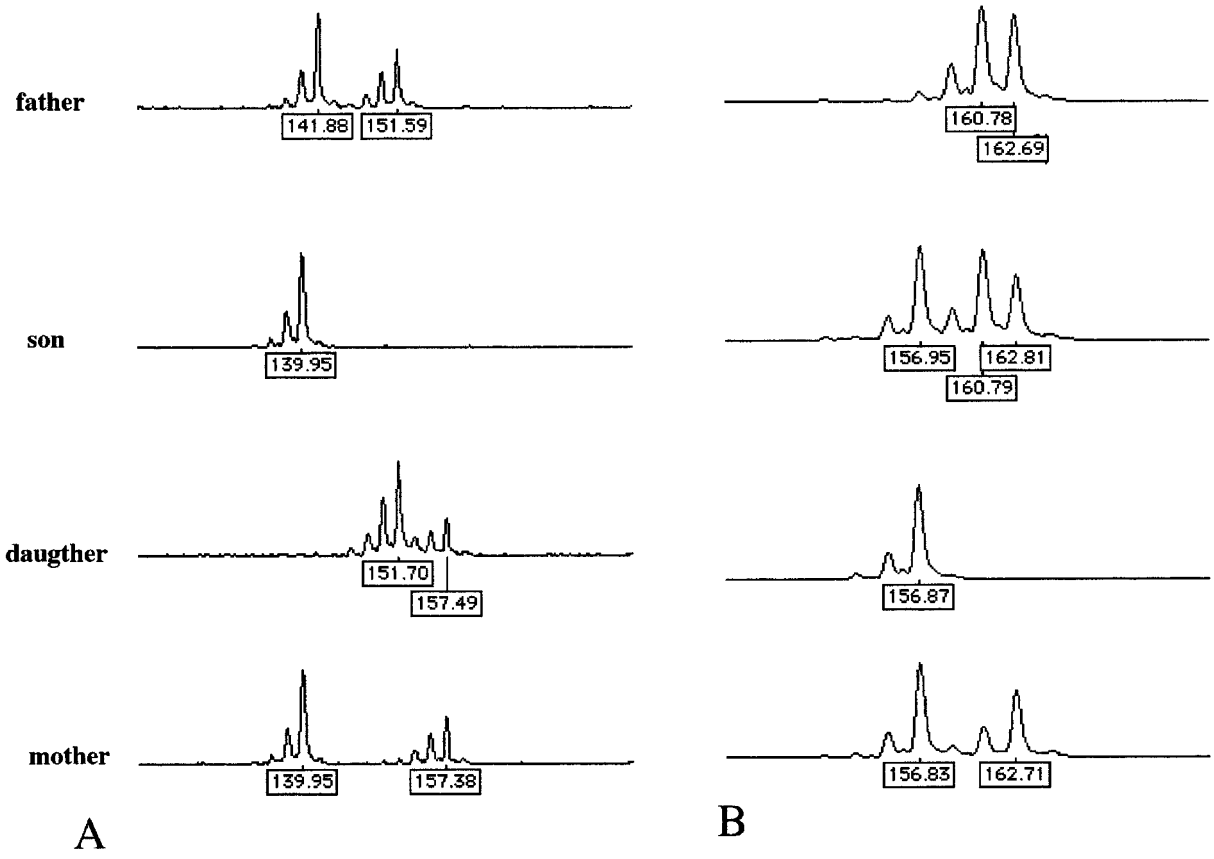

Figure 2 Identification of a telomeric rearrangement in family 1. Genotyping data of the four members of family 1 typed with D6S281, **A** and D10S1676, **B**. The results show that the son failed to inherit the paternal chromosome 6 allele and is trisomic for the chromosome 10 marker. His sister is missing the paternal allele for the chromosome 10 markers.

Table 4 Genotype analysis with chromosome 6, 10, 16 and 17 markers

Family 1						
Marker	*Location*	*Father*	*Son*	*Daughter*	*Mother*	
D10S1700	10q26.3	130/132	130/132	**128**	128/130	
D10S212	10q26.3	188/194	**188/194/196**	**194**	194/196	
D10S1676	10q26.3	161/163	**157/161/163**	**157**	157/163	
D10S217	10q26.2	203	197/203	**197**	197/201	
D6S281	6q27	142/152	**140**	152/158	140/158	
D6S1719	6q27	173	**177**	173/183	177/183	
D6S1599	6q27	130/132	130/132	130/132	130/132	
Family 2						
Marker	*Location*	*Father*	*Son*	*Mother*		
D16S3121	16q24.3	76	**76**	78		
D16S3026	16q24.3	204/206	204/206	204		
D16S3023	16q24.3	73/85	73/87	85/87		
Family 3						
Marker	*Location*	*Father*	*Child 1*	*Child 2*	*Child 3*	*Mother*
D10S1700	10q26.3	129	**127**	**127**	127/129	127/129
D10S212	10q26.3	188/194	194	194	188/194	194/198
D10S169	10q26.2	96/104	96/110	104/110	104/110	108/110
Family 4						
Marker	*Location*	*Father*	*Son*	*Mother*		
D17S928	17q25.3	89/95	**83/97**	83/97		
D17S914	17q25.3	203	**195**	195		
D17S2200	17q25.3	235	235	235		
D17S784	17q25.3	228/234	228/232	232		
D17S1830	17q25.3	108/110	110/112	112/114		

Markers used are shown on the left and size of the different alleles are given in base pairs for the parents and the affected child. Genotypes informative for monosomy/trisomy or maternal disomy are indicated in bold underlined characters.

map database. Our data clearly demonstrate that the two children have inherited opposite derivatives of a balanced paternal translocation t(6;10)(q27;q26), resulting in a monosomy 6q27/trisomy 10q26 in the son and in a monosomy 10q27 in the daughter (Figure 2B and Table 4). The rearrangement extends up to the D6S1719 locus on chromosome 6 and up to the D10S217 locus on chromosome 10 (Table 4). Based on the marker position, we estimated that the rearrangement involves a region of at least 15 cM on chromosome 6 and 18 cM on chromosome 10. FISH analysis confirmed the balanced rearrangement in the father and demonstrated that the daughter is trisomic for the region 6q27 (Figure 3). Since the genotype of the daughter at the locus D6S281 is normal, a crossing over between the translocated part of the chromosome 6 and the normal chromosome 6 must have occurred (Figure 4). The crossing over should have occurred during meiosis I in the carrier father so that translocated part of the chromosome 6 on the der(10) was exchanged with the corresponding part of the intact chromosome 6 copy.

Chromosome 16q anomaly in family 2

In family 2, a maternal non contribution was detected at the D16S3121 locus on chromosome 16q while flanking markers were regularly inherited (Table 4). FISH analysis using chromosome 16q probes detected two normal signals in all metaphase spreads examined (data not shown). Since non-paternity was clearly excluded, this result could be accounted for by either an allele mutation in the child (so that the final repeat length is the same as length of the father allele) or a small deletion undetectable using the Cytocell probes.

Monosomy 10qter in family 3

In family 3, two severely affected children (cases 1 and 2) inherited a single maternal allele and no paternal allele at the D10S1700 locus on chromosome 10q while a mildly affected child (case 3) inherited one allele from each parent (Table 4). Genotyping the rearrangement with additional markers revealed that the breakpoint maps distal to D10S169 so that the deleted region is less then 3 cM in size. FISH analysis of this family was unremarkable, a feature which should be related to the proximal location of the FISH probes with respect to the breakpoint. In the absence of any evidence for segmental trisomy in the third child, and because of the results of FISH analyses, we are presently unable to discriminate between a germinal mosaicism for a paternal deletion or the unbalanced inheritance of a balanced paternal translocation.

Maternal heterodisomy in family 4

The proband of family 4 has failed to inherit a paternal allele at the D17S928 locus but carried a maternal heterodisomy for

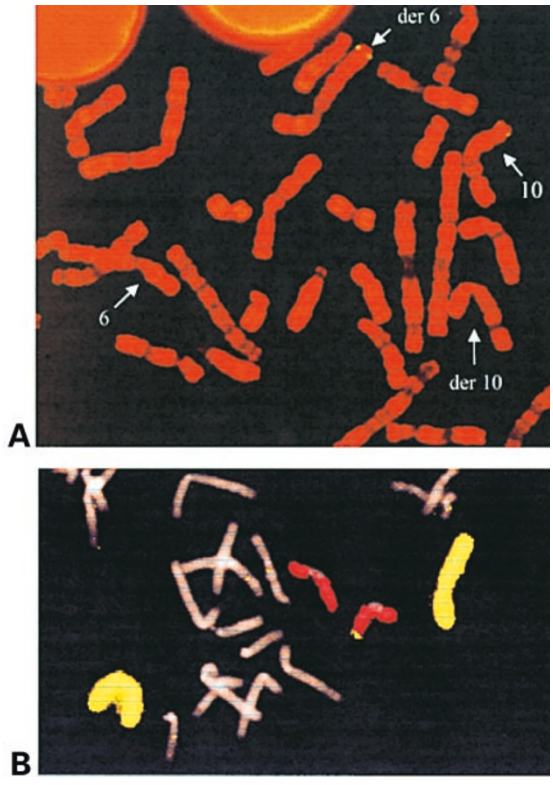

Figure 3 FISH studies in family 1. (**A**) FISH analysis was performed with a PAC probe containing marker D10S217 and revealed a balanced translocation in the carrier father. Two signals were present which correspond to the normal chromosome 10 homologue or to the derivative chromosome 6. The two other unlabelled normal chromosome 6 and derivative chromosome 10 are also indicated. (**B**) Whole chromosome painting using chromosomes 6 and 10 probes on chromosome of the daughter confirms the presence of an unbalanced karyotype.

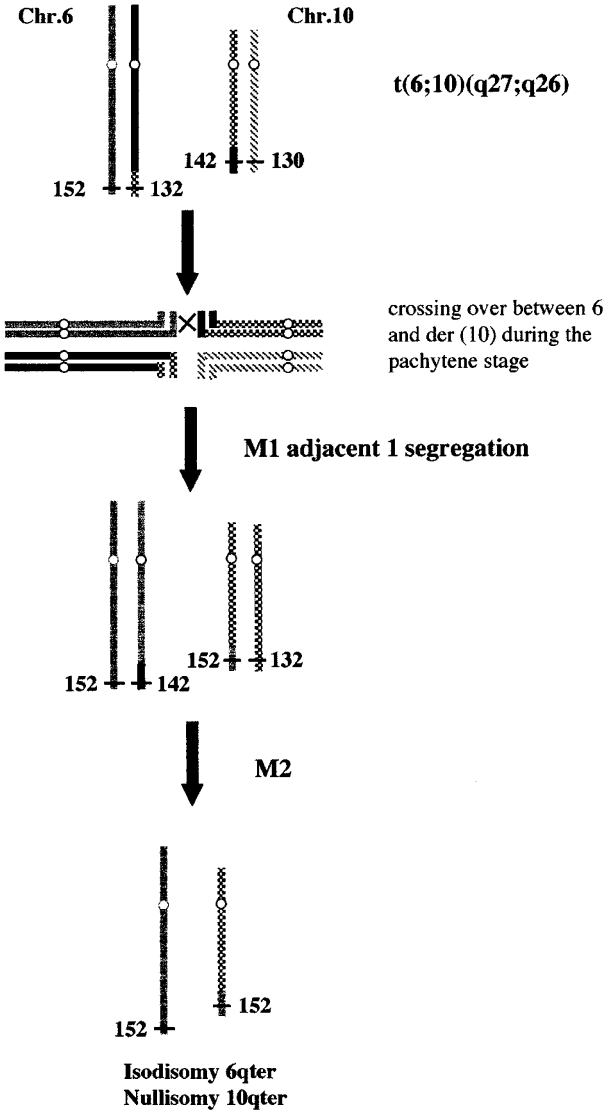

Figure 4 Proposed mechanism of the recombination event leading to the genotype of the daughter in family 1. A crossing over must have occurred during the pachytene stage, when the two translocated chromosomes and their normal counterparts come together as a quadrivalent. After adjacent 1 segregation and the second division, this recombination leads to isodisomic 6qter/nullisomic 10qter gamete. The genotypes for the D10S1700 and D6S281 loci are indicated.

chromosome 17q markers (Table 4). Genotyping of additional markers confirmed the absence of paternal contribution for this region and indicated that the extent of the maternal disomy was smaller than 11 cM. Analysis of the propositus and his parents with STSs from other chromosomes showed typical mendelian inheritance. A detailed analysis of this case will be described elsewhere (Rio *et al*, personal communication). Since uniparental disomies (UPD) are frequently associated with confined placental mosaicism, we cannot conclude whether the abnormal phenotype results from the dosage of an imprinted gene or from mosaicism for trisomy 17q.

Discussion

Here, we report on a novel and efficient automated strategy based on the fluorescent genotyping technique to screen for cryptic telomeric rearrangements in children with developmental delay and multiple congenital anomalies. Despite normal conventional karyotypes in the 29 probands, we identified three chromosomal abnormalities in our series (about 10%). The prevalence of subtle telomeric abnormalities was first estimated to 7.4% using cytogenetic detection methods.[10] A subsequent study based on the screening for submicroscopic rearrangements using microsatellite markers suggested a higher frequency (18%) and concluded that genotyping might be more sensitive than previously used

FISH techniques.[13] Our results give additional support to this conclusion. Moreover, in contrast to cytogenetics (which requires a high technical expertise), genotyping offers the advantage of giving quantitative and objective results that can be automated in many respects. Lastly, genotyping can directly identify the parental origin of the rearrangement.

The set of markers described here have an average heterozygosity score of 0.75. Since only few chromosome maps encompass telomeres, several loci tested (Table 2) are likely to lie hundreds of kilobases away from the telomere. Sequence data derived from the human genome sequencing projects will hopefully allow us to define more informative and/or telomeric microsatellite markers, so that the primary sets of markers described here will be improved. The development of new fluorophores will also allow reduction of the number of sets. We estimated extensive genotyping of one child and his two parents to cost 80 dollars, ie three to four times less than the cost estimated for the cytogenetic method,[10] and progress in automation is likely to make this approach even more affordable.

However, it should be noted that this approach also has its limitations. First, it cannot detect balanced rearrangements when the dosage is unaltered. A second limitation is that dosage differences cannot be reliably detected. Hence, this technique may overlook cases of monosomy and trisomy when the parents share the same allele or cases of small tandem duplications. Finally, this screening technique may generate false-positive results due to the instability of microsatellites, as probably observed in family 2. Thus, non mendelian inheritance of a single microsatellite by itself does not provide evidence that a chromosomal rearrangement has occurred. Genotyping of additional markers and/or FISH analyses are required to support this conclusion. In addition, FISH analyses are required to distinguish a *de novo* rearrangement from an inherited chromosome imbalance and to identify the carrier(s) of the balanced anomaly in the family.

Finally, the microsatellite technique provides the unique opportunity to detect uniparental disomies, a major disease causing mechanism.[18] This feature is illustrated by the results of family 4 where the proband carries a maternal heterodisomy of the telomere of chromosome 17q. On the basis of the haplotype analysis, the mechanism responsible for this partial UPD of chromosome 17 is likely to be the consequence of the fertilization of a maternal disomic gamete (due to a nondisjunction error) by a monosomic paternal gamete. Postzygotically, a recombination must have occurred between one of the maternal chromosome 17 and the paternal one. Subsequently, and independently, the maternal chromosome 17 has been lost in the progenitor cell line. In this family, the abnormal phenotype could result from either confined placental mosaicism or the imprinting of this part of the genome. Placental tissues were unavailable in this case so we could not test for trisomy 17 in these cells. However, confined placental mosaicism is usually associated with intrauterine/post-natal growth retardation, a feature which was not observed in the proband. On the other hand, maternal isodisomy for chromosome 17 has previously been described in a 2-year-old boy with normal growth and psychomotor development,[19] and the terminal long arm of chromosome 17 is not known to undergo imprinting.[20] Further investigations are therefore required to know whether this region contains one or several imprinted gene(s).

Finally, in addition to its clinical relevance, this novel strategy will hopefully allow the delineation of new contiguous gene syndromes and the identification of new imprinted regions.

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