Subtelomeric Chromosome Rearrangements in Children with Idiopathic Mental Retardation: Applicability of Three Molecular-cytogenetic Methods

Alenka Erjavec-Škerget, Špela Stangler-Herodež, Andreja Zagorac, Boris Zagradišnik, Nadja Kokalj-Vokač

Medical Genetics Laboratory,
Maribor Teaching Hospital,
Maribor, Slovenia

Aim To identify cryptic subtelomeric rearrangement, a possible cause of idiopathic mental retardation by means of multiprobe telomere fluorescent in situ hybridization (T-FISH).

Methods Hundred patients (median age 3.0 years) with mental retardation and dysmorphic features were screened using specific T-FISH probes. Multiplex ligation-dependent probe amplification and comparative genomic hybridization were used for the confirmation of results.

Results Telomere fluorescent in situ hybridization revealed 11 subtelomeric abnormalities in 10 patients (10%; 95% CI, 5.0-17.5). Four of these had only a deletion of subtelomere 2q, which was apparently a normal variant. Among 6 true aberrations (6%; 95% CI, 2.5-12.5) we found 2 de novo subtelomeric deletions and 4 unbalanced subtelomeric rearrangements (one de novo). All clinically significant subtelomeric rearrangements were confirmed by multiplex ligation-dependent probe amplification. Comparative genomic hybridization was used to investigate the whole genome of patients in whom a subtelomeric anomaly was found, confirming some, but not all subtelomeric rearrangements.

Conclusion Telomere fluorescent in situ hybridization and multiplex ligation-dependent probe amplification are both very useful and interchangeable methods for the detection of unbalanced chromosome rearrangements, but T-FISH also detects balanced rearrangements. In our experiment, the resolution power of comparative genomic hybridization was too low for subtelomeric screening compared with T-FISH and multiplex ligation-dependent probe amplification.

> **Correspondence to:**

Alenka Erjavec Škerget
Medical Genetics Laboratory
Maribor Teaching Hospital
Ljubljanska 5
SI-2000 Maribor, Slovenia
alenka.erjavec@sb-mb.si

> **Received:** June 5, 2006
> **Accepted:** July 4, 2006

> **Croat Med J. 2006;47:841-50**

A significant diagnostic challenge exists to identify the new causes of mental retardation. Mental retardation is present in about 1-3% of individuals in the general population, but it can only be explained in about half of the cases, despite thorough clinical and laboratory investigations (1). Several lines of evidence indicate that genetic factors are involved in many of the idiopathic cases, as they often show prenatal and postnatal signs such as dysmorphic features, growth retardation, and malformations or have a family history of mental retardation (1).

The subtelomeric regions are interesting from a genomic perspective, as they are gene rich and often involved in chromosomal rearrangements (2). Most telomeres stain lightly with G-banding, and small rearrangements are therefore difficult to detect. In 1996, a complete set of fluorescence in situ hybridization (FISH) probes located within a distance of 300 kB from the telomeric repeats was presented, and an updated set was announced (3,4). These probes made it possible to analyze all chromosome ends for subtelomeric rearrangements in one experiment (5). Today, the most frequently used methods to screen for chromosomal abnormalities in patients with mental retardation arethe following: FISH with a complete set of subtelomere probes (T-FISH) (5), multicolor FISH/spectral karyotyping (M-FISH/SKY) (6,7), multiplex amplifiable probe hybridization (MAPH) (8), multiplex ligation-dependent probe amplification (9), comparative genomic hybridization (10), high resolution-comparative genomic hybridization (11), microarray-comparative genomic hybridization (12-14), primed in situ labeling (PRINS) (15), and genotyping (1). The prevalence of abnormalities in the entire group of patients with idiopathic mental retardation is 5.1% but the figure is higher (6.8%) in the individuals with moderate to severe mental retardation (16).

We analyzed 100 patients with mental retardation and/or dysmorphic features using T-FISH. By means of multiplex ligation-dependent probe amplification, positive results of T-FISH were confirmed and by means of comparative genomic hybridization the whole genome of patients with subtelomeric aberrations was screened.

Patients and methods

Patients

We screened 100 patients, 54 girls and 46 boys aged 0-19 years (median age 3.0) (Figure 1). Patients were selected according to the following criteria (1): mild to moderate mental retardation (IQ<70) or developmental delay with dysmorphic features (2); normal karyotype at the level of resolution >450 bands, except in one case (case 3), in which the source of the translocated material was confirmed using T-FISH and one subtelomeric deletion was found (3); exclusion of other possible etiologies by a full genetic assessment and relevant tests, for example, for Fragile X syndrome. Children aged 0.1-6 years (60%) were investigated because of dysmorphic features and children aged 6.1-19 years (84%) were investigated because of idiopathic mental retardation. Ethical approval was granted by the National Medical Ethics Committee of Slovenia (No. 64/05/04).

Methods

Cytogenetic and multiprobe fluorescent in situ hybridization. Metaphase slides were prepared

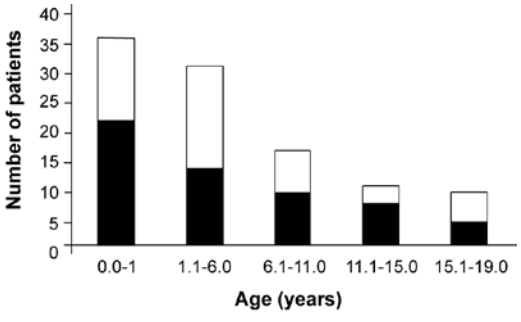

Figure 1. Age and gender of patients included in the study. Open bars – males, closed bars – females.

Table 1. Locus specific FISH probes, their characterization, and results on derivative chromosomes in patients with clinical significant subtelomeric rearrangements*

Case	Probe region	Probe name	Gene marker	FISH results on derivative (der) chromosome
Case 1 (F)	2q37.3	YAC 762G3	D2S2042	der(2): one signal
	Xp22.33	PAC dJ617A9	AC005295	der(X): no signal
	Xp22.33	BAC bA155F12	AC027389	no signal
	Xp22.33	BAC bA751F9	DXYS104 (AC068583,2)	no signal
	Xp22.33	BAC bA131H1	AC073615	no signal
	Xp22.33	BAC bA215A12	AC084716	no signal
	Xp22.3	YAC 742C6	AFMB290XG5	no signal
	Xp22.31	BAC bA483M24	AC073583	no signal
	Xp22.31	BAC bA126022	AC073488	no signal
	Xp22.31	BAC bA382D24	ends†	one signal
Case 2 (M)	10q23.3	RP11-402D21	AL359198	der (13): no signal
	10q24.1	RP11-7D5	AL356632	no signal
	10q24.1	RP11-34E5	AL138765	one signal
	10q24.2	RP11-548K23	AL355315	one signal
	13q33.3	RP11-15L1	AL138914	one signal
	13q34	RP11-120J20	AL157875	one signal
	13q34	RP11-75F3	AL136032	no signal
	13q34	RP11-245B11	AL161774	no signal
	13q34	RP11-569D9	AL160396	no signal
Case 3 (M)	Xp22.33	PAC dJ617A9	AC005295	der (X): no signal
	Xp22.33	BAC bA155F12	AC027389	no signal
	Xp22.33	BAC bA751F9	DXYS104 (AC068583,2)	no signal
	Xp22.33	BAC bA131H1	AC073615	no signal
	Xp22.33	BAC bA215A12	AC084716	no signal
	Xp22.3	YAC 742C6	AFMB290XG5	no signal
	Xp22.31	BAC bA483M24	AC073583	no signal
	Xp22.31	BAC bA126022	AC073488	one-split signal
	Xp22.31	BAC bA382D24	ends†	one signal on Xp
	Xq28	PAC dJ231B19	NA	one signal on Xq
	Xq28	RP11-157E12	AF282854	one signal on Xq
	Xq28	RP11-402H20	AC016977	one signal on Xq
	Xq28	YAC 810M4	AC027048	one-split signal
Case 4 (M)	8q24.22	BAC bA269I24	AC090987	der(21): no signal
	8q24.22	RP11-21D15	AC019342	no signal
	8q24.3	YAC774G12	D8S1741	one signal
	21q22	YAC886G8	D21S266	one signal
	21q22.3	RP11-433E24	ends†	no signal
Case 5 (F)	9p24.1	bA31M2	AL158147	der(9): no signal
	9p24.1	bA125K10	AL133283	no signal
	9p24.1	bA472F14	AL353718	no signal
	9p24.1	bA77E14	AL354694	no signal
	9p23	bA50C21	AL356054	one signal
Case 6 (F)	10q26.3	RP11-108K14	AL161645	der(11): one signal
	10q26.3	RP11-408L20	AC067746	one signal
	10q26.2	RP11-333H4	AL160174	one signal
	10q26.2	RP11-21M8	AL15771	one signal
	10q26.2	RP11-16P8	AL583860	one signal
	10q26.13	RP11-359B3	AL929444	one signal
	10q26.13	RP11-564D11	AC073585	no signal
	11q25	RP11-265F9	ends†	no signal
	11q25	RP11-828F10	AP001110	no signal
	11q25	RP11-90A13	ends†	no signal
	11q24.3	RP11-535N6	AP004371	no signal
	11q24.3	RP11-589G12	AP003782	no signal
	11q24.2	RP11-480C22	AC074180	no signal
	11q24.2	RP11-15J15	ends†	one signal
	11q24.2	RP11-417F7	AP003069	one signal

*Abbreviations: M – male; F – female; NA – not available.
†Determined by bacterial artificial chromosomes-end (BAC-end) sequencing.

from a lymphocyte culture of peripheral blood as described previously and all patients were karyotyped using classical cytogenetic techniques (17). Following the manufacturer's protocol, the Chromoprobe Multiprobe T System (Cytocell, Ltd, Banburry, UK) was used to detect subtelomeric regions.

A minimum of five metaphases was analyzed for each chromosome. More than 10 metaphases and 10 interphases were analyzed when a chromosome rearrangement was detected. The slides were analyzed in a Zeiss Axioplan fluorescence microscope (Zeiss, Götingen, Germany) and images were captured by a cooled CCD camera (Cohu, San Diego, CA, USA). The Applied Imagine software system was used (Applied Imaging, Newcastle, UK) (5).

Abnormal FISH results were confirmed with locus-specific subtelomeric probes derived from yeast artificial chromosomes (YACs), P1 artificial chromosomes (PACs), and bacterial artificial chromosomes (BACs) which were a kind gift from Dr M. Rocchi, University of Bari, Italy (Table 1).

Multiplex ligation-dependent probe amplification. The method was performed as previously described (9,18) using a commercial kit SALSA P036 for multiplex ligation-dependent probe amplification assay (MRC, Amsterdam, Holland) according to the manufacturer's protocol (*www.mrc-holland.com/mlpa_info.html*). Samples were analyzed using a Beckman CEQ 8000 capillary electrophoresis system (Beckman Coulter, Fullerton, California, USA).

Microsoft Excel software 2002 (Microsoft, Seattle, WA, USA) was used to record peak areas corresponding to the signal from each probe. In order to produce normalized ratios reflecting the relative probe dosage, each peak area was divided by the sum of all peaks. For each probe, this ratio was divided by the same ratio from an unaffected control run in the same experiment. Expected normalized values were 1.0 in the absence of copy number change, and 0.5 and 1.5 in the case of heterozygous deletion and duplication, respectively.

Comparative genomic hybridization. The method was performed as described previously (19). Briefly, the patient's DNA and normal reference DNA were labeled with Spectrum Green and Spectrum Orange (Vysis, Downersgrowe, Ilinois, USA). A total of 1 μg of DNA and 30 μg Cot1 DNA were hybridized to normal metaphase chromosomes. Slides were hybridized for three days, washed, and counterstained with 4,6-diamidino-2-phenylindole (DAPI). Comparative genomic hybridization image capture was performed with a Cytovision (Applied Imaging, Sunderland, UK) interfaced to a fluorescence microscope Axioplan (Zeiss, Gotingen, Germany). In each case, at least 10 metaphases were analyzed. Karyotyping was performed based on the DAPI-banding pattern. Green and red fluorescence intensities were measured along the length of each homologue chromosome. The average green to red ratio fluorescence intensity ratio profile was calculated for each chromosome. In regions of normal sequence copy numbers, the average green to red ratio was found to be around 1.0. Chromosomal regions with a ratio above 1.2 were considered to be gained, whereas regions with a ratio below 0.8 were considered to be lost.

Results

Frequency with T-FISH

Testing with the T-FISH method revealed subtelomeric chromosomal rearrangements in 10 of 100 proband patients (10%; 95% CI 5.0-17.5), where 11 independent subtelomeric rearrangements were found. Among them, 4 patients had an unbalanced cryptic rearrangement, 2 patients had a deletion of the subtelomeric region (one of whom had 2 subtelomeric deletions – one clinically significant and one polymorphism-deletion of region 2qter), while 4 patients showed only an apparently normal variant of 2qter deletion. The

frequency of clinically significant subtelomeric aberrations was 6% in our study population (95% CI 2.5-12.5), when the cases with 2qter polymorphism were excluded.

Multiplex ligation-dependent probe amplification confirmed all clinically significant subtelomeric rearrangements. In contrast to T-FISH, multiplex ligation-dependent probe amplification failed to detect 2qter polymorphism.

With comparative genomic hybridization we confirmed subtelomeric anomalies larger than 8Mb: dim(Xpter) in case 1; enh(10q23.3->qter) in case 2; dim(Xpter) in case 3; enh(8qter) in case 4, dim(9pter) in case 5, and in case 10 enh(10qter) and dim(11qter).

Aberrations and clinical features of patients

The karyotypes, clinical features, and parental studies of the abnormal cases are described, combining the results of T-FISH, locus-specific FISH, multiplex ligation-dependent probe amplification, and comparative genomic hybridization.

Case 1: 46,XX. ish del(X)(pter-)(bA126O2 2-), del(2)(qter-)(D2S2986-). With T-FISH we found the deletion of region 2qter, which was not clinically significant (Table 1) and a de novo deletion of the Xpter region (Figure 2A). A larger deletion on the short arm of the X chromosome was confirmed using several probes covering the Xp22.33 and Xp22.31 regions (Table 1). The breakpoint was determined to be between probes bA126O22 and bA382D24, which represents an approximate 9Mb deletion. Both parents had normal X chromosomes, while the father had the same 2qter polymorphism as his daughter. Multiplex ligation-dependent probe amplification (Figure 2B) and comparative genomic hybridization (Figure 2C) confirmed the deletion of chromosome Xpter, but not the polymorphism.

This girl with dysmorphic features was the first child of phenotypically normal parents. Her birth and birth-weight were normal. Growth retardation was observed at eight months. Clinical features included: micrognathia, high palate, broad forehead, flat nasal bridge, hypertelorism, hydronephrosis, and vesicoureteric reflux.

*Case 2: 46,XY,der (*13*). ish der(13)t(13;10) (qter-,q23.3→qter+).* Case 2 has already been described (21). With T-FISH and a set of BAC probes we determined the breakpoint on 10q23.3 between BAC's RP11-402D21 and RP11-7D5 and on 13q34 between BAC's RP11-120J20 and RP11-75F3 (Table 1). The case was reexamined using the comparative genomic hybridization and multiplex ligation-dependent probe amplification techniques. The deletion on 13q was not confirmed by comparative genomic hybridization, while the trisomic 10q was clearly seen. Multiplex ligation-dependent probe amplification detected monosomy of 13q and trisomy of the10q subtelomeric region.

The patient had psychomotor and growth retardation, dysmorphic facial features, toe anomalies, right choanal hypoplasia, coloboma, genital anomalies, bilateral renal hypoplasia, and early chronic renal failure.

Case 3: 46,XY, inv(9)(p11q13). ish rec(X) (qter +, pter-)(bA126O22-, YAC810M4+). Case 3 has already been described (20) but now the case was reexamined using multiplex ligation-dependent probe amplification and comparative genomic hybridization. Cytogenetic and multiprobe fluorescent in situ hybridization and a set of BAC probes revealed the unbalanced rearrangement in bA1269022 on the Xp22.3 region and in bA810M4 on the Xq28 region (Table 1). Multiplex ligation-dependent probe amplification revealed a deletion of Xpter and the duplication of the Xqter region. Only the deletion of Xpter was detected by comparative genomic hybridization, while trisomy of the Xqter region was not seen.

The patient had severe growth and developmental delay, ichthyosis, hypogonadism, limb shortness, hypoplasia of the corpus callosum, a round flat face, and a thin upper lip as a conse-

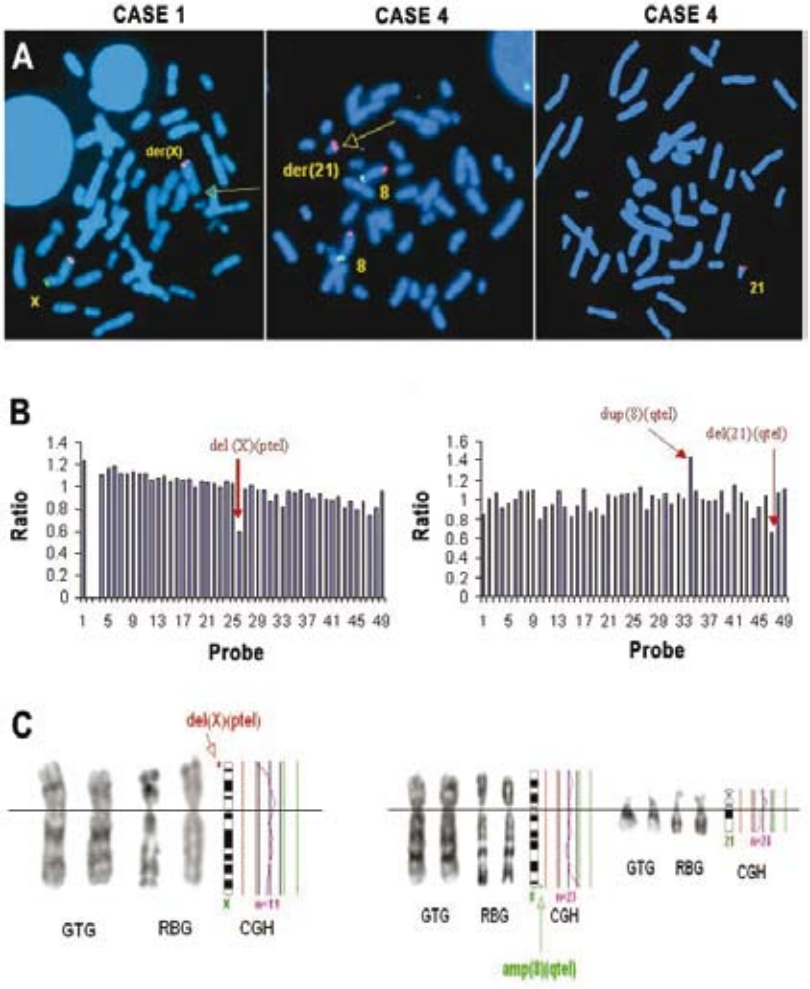

Figure 2. Two cases (case 1 and 4) showing subtelomeric anomalies detected either by multiplex ligation-dependent probe amplification, partial G-banding with tripsin and Giemsa (GTG) and R-banding with heat and Giemsa (RHG) banding, comparative genomic hybridization and T-FISH (Multiprobe T-System) (green signals for p-ter and red signals for q-tel). (**A**) T-FISH result of chromosome X in case 1 (left panel), showing the absence of one Xpter signal. In case 4, T-FISH of chromosomes 8 (middle panel) shows an additional signal on der (21); T-FISH of chromosome 21 shows the absence of one 21pter signal (right panel). (**B**) Multiplex ligation-dependent probe amplification showing a del(X)(ptel) in case 1 (left panel) and dup(8)(qtel) and a del(21)(qtel) in case 4 (right panel). (**C**) GTG and RBG banding of chromosomes. In case 1 (left panel), banding and comparative genomic hybridization and comparative genomic hybridization (CGH) shows a del(X)(ptel) on chromosome X. In case 4 (right panel), amplification of chromosome 8 [amp(8)(qtel)] was detected, but not the deletion of chromosome 21.

quence of a subtelomeric deletion of the chromosome X (STS, SHOX, ARSE, and KAL genes) and duplication of complete region Xq28.

Case 4: 46,XY. ish der(21)t(21;8)(qter-,qter+) (YAC886G8-,RP11-21D15+). After testing with T-FISH, an additional signal of the 8q subtelomere region on a G-group chromosome and the absence of one signal of the 21q subtelomere region were noticed (Figure 2A). Fluorescent in situ hybridization results from different BACs and YACs determined the breakpoint on 8q24.23 between BAC RP11-21D15 and YAC 774G12 and on 21q22.3 between YAC 886G8 and BAC RP11-433E24 (Table 1). Multiplex ligation-dependent probe amplification confirmed the same results as T-FISH: deletion of the subtelomere on chromosome 21q and trisomy of the subtelomere on chromosome 8q (Figure 2B). Approximately 8Mb of material of chromosome 8 was trisomic (confirmed by comparative genomic hybridization – Figure 2C and 3.9Mb of material of chromosome 21 was missing (not de-

tected by comparative genomic hybridization). A balanced translocation with the same breakpoints on chromosomes 8 and 21 was found in the patient's father.

Mongoloid slant of the eyes was the only dysmorphic feature observed in this patient. Early development was delayed: he sat at the age of one year, walked at 18 months of age, and spoke at the age of 4 years. He has suffered from epileptic seizures since the age of 30 months. He was obese and clumsy and had poor fine motor skills. He had borderline intelligence and problems with concentration, but finished primary school.

Case 5: 46,XX. ish del(9)(pter-)(23→pter-). Using T-FISH we found a deletion of the 9pter subtelomeric region. With locus-specific BAC probes the breakpoint of the deletion, which occurred de novo, was identified. RP11-50C21 (9p23) was the most terminal probe on the derivative chromosome 9 (Table 1), which means that the whole 9p24 region was missing (about 10Mb). The deletion was confirmed by multiplex ligation-dependent probe amplification and comparative genomic hybridization.

This female infant was born after a normal pregnancy to healthy, unrelated parents. She had dysmorphic features, including epicanthal folds and mongoloid slanting eyes, and suffered from epilepsy.

Case 6: 46,XX. ish der(11) t(11;10)(qter-, qter+)(RP11-15J15-,RP11-359B3+). Using T-FISH we revealed three red signals for subtelomeres of chromosome 10 and one red signal for subtelomeres of chromosome 11. With locus specific FISH, we confirmed them and determined the size of subtelomeric abnormality (Table 1). The chromosome 10 was trisomic of the segment 10q26.13→10qter and the chromosome 11 had a deletion of the segment 11q24.2→11qter. Using multiplex ligation-dependent probe amplification and comparative genomic hybridization we confirmed the results. On the derivative chromosome 11, approximately 8Mb of chromosomal material was deleted and replaced with 8.3Mb material of chromosome 10. The results of subtelomeric FISH in patient's father showed a balanced translocation: 46,XY. ish t(10;11)(qter-, pter+;qter-,pter+).

The patient was a 17-year-old girl with mild mental retardation, some facial and skeletal dysmorphic features, aplastic anemia, and thrombocytopenia.

Discussion

We report on the use of 3 different molecular cytogenetics methods, T-FISH, multiplex ligation-dependent probe amplification, and comparative genomic hybridization in a cohort of patients with mental retardation, dysmorphic features, and a normal cytogenetic analysis, using G-bands with tripsin and Giemsa (GTG) and R-bands with heat and Giemsa (RHG) banding techniques (with the exception of one case). In our series of 100 patients, 11 subtelomeric aberrations were detected in 10% of the patients with T-FISH technique.

Based on the summarized data of 20 studies, the overall rate of subtelomeric anomalies in idiopathic mental retardation averages 5.1%, ranging from 0 to 35.7%, with an incidence of 3 to 9% found in the majority of studies (22). The largest study about prevalence of subtelomere rearrangements detected with T-FISH, presents data compiled from 11 688 cases: the detection rate of clinically significant abnormalities was 2.5% with an additional 0.5% detection rate of polymorphic variant (23).

In our study 5% of patients had the deletion of region 2qter (locus D2S2986), none of which was confirmed by locus-specific FISH or multiplex ligation-dependent probe amplification. Parental studies were performed in two cases, with both deletions being inherited from phenotypically normal parents. A polymorphism at the 2q subtelomere was first identified by Macina et al (24). Since then, the deletion of 2qter has been reported in study populations with a frequency

of 1.5 to 8.2% and an average frequency of 5% (6,25,26). Based on our results, the segregation analysis of the 2qter deletion in family members in two cases showed no correlation with mental retardation or other phenotypic anomalies. Our observations support the conclusion that the deletion of locus D2S2986 in the 2qter region is a common variant, with this clinically significant result being interpreted as a polymorphism or a normal variant. Multiplex ligation-dependent probe amplification did not detect this polymorphism because 2qter probe for multiplex ligation-dependent probe amplification subtelomeric kit P036 is located outside the polymorphic 2q subtelomeric region.

Our study also compared the usefulness of three different methods, T-FISH, multiplex ligation-dependent probe amplification, and comparative genomic hybridization for detecting subtelomeric anomalies in clinical diagnostics.

The usefulness of T-FISH for screening for chromosomal anomalies in mentally retarded patients has been well demonstrated (4,27). This method has the advantage of instant detection of deletions and balanced or unbalanced translocations, which can be located. Mosaic cases can also be detected by T-FISH, which is not always possible with the other two methods (9,19). Potential concerns over using T-FISH are that 11 probes can cross hybridize with other chromosome regions (24,26). Thus, this method emphasizes the necessity of follow-up studies on parents or other relatives for clinical interpretation of positive FISH findings. A third generation of the Chromoprobe Multiprobe System (Cytocell) has been developed, which has a 2qter BAC probe with no polymorphism. The detection rate of all detected subtelomeric abnormalities with third generation of DNA probes is therefore lower than in previous studies (23).

For screening purposes, T-FISH method is expensive and time consuming. Therefore we sought a faster and cheaper method for detecting subtelomeric deletions and amplifications. Multiplex ligation-dependent probe amplification is a new method for the measurement of gene copy number that holds great promise for detection of gene abnormalities (9). We applied multiplex ligation-dependent probe amplification for subtelomeric testing by comparing it with the T-FISH and comparative genomic hybridization techniques. Multiplex ligation-dependent probe amplification confirmed all clinical significant subtelomeric rearrangements. Based on our results there was a 100% concordance between these two techniques (T-FISH and multiplex ligation-dependent probe amplification) when the polymorphisms were excluded. The multiplex ligation-dependent probe amplification assay is a simple, fast, sensitive, and specific technique. Its characteristics are suitable especially for high-throughput screening or testing. A disadvantage of the multiplex ligation-dependent probe amplification method is the fact that confirmation and location by FISH is still necessary. Recently, 210 patients with idiopathic mental retardation were tested with multiplex ligation-dependent probe amplification and the detection rate of 6.7% was reported (28). Clinical relevant abnormalities occurred in 6.3%, 5.1%, and 1.7% of the mildly, moderately, and severely retarded patients. In contrast to other screening studies, their highest detection rate was among the mildly retarded, emphasizing the importance of screening subtelomeric aberrations in this group of patients (29).

Comparative genomic hybridization provides a genome-wide screening for chromosomal imbalances in a single hybridization directly from DNA samples, without requiring the sample material to be mitotically active (19). The main disadvantages of comparative genomic hybridization include its inability to detect balanced rearrangements or regions consisting of highly repetitive sequences and its inability to detect the small amount of pathogenic cells. With comparative genomic hybridization we confirmed subtelomeric anomalies larger than 8Mb. Our study shows that comparative genomic hybridization

has the potential to diagnose aberrations that are difficult or impossible to detect even with good quality R- and G-banding techniques, but compared with subtelomeric screening with T-FISH and multiplex ligation-dependent probe amplification, its resolution is too low.

The resolution of comparative genomic hybridization has been reported to be in the range of 5-10Mb (10), 4-5Mb (16), and, by high resolution comparative genomic hybridization, 2-3Mb (11,30). First screening approach to detect subtle chromosome rearrangements in mental retardation patients with dysmorphic features was carried out with a resolution of 5 Mb (31). High resolution comparative genomic hybridization was suited for routine screening for cryptic chromosomal imbalances throughout the whole genome. The detection rate in patients with mental retardation was 12% (32).

The resolution in array-based comparative genomic hybridization is increased significantly, as theoretically the detection of unbalanced aberrations on the size of the single BAC clone is possible (12). The similar detection rate as by high resolution-comparative genomic hybridization was also found with it: 8 to 10% of the patients had clinically relevant chromosomal rearrangements (33,34).

Based on our results, we concluded that T-FISH and multiplex ligation-dependent probe amplification were very useful, efficient, and interchangeable methods, with some exceptions, while the resolution by comparative genomic hybridization was too low for screening subtelomeres. Multiplex ligation-dependent probe amplification is a method that combines simplicity with high sensitivity (9), but some important facts remain unknown (balanced rearrangements, location, mosaicism). Therefore, T-FISH and multiplex ligation-dependent probe amplification methods cannot always be interchangeable as it was previously reported (35). Multiplex ligation-dependent probe amplification is the method of subtelomeric screening, which is suitable especially for high-throughput screening. In combination with T-FISH, which could be used for result confirmation, it may be the method of choice for routine cytogenetic diagnostics. For sporadic diagnostic cases, T-FISH is the most suitable method.

Until the genome-wide screening techniques, like array-comparative genomic hybridization are more accessible for routine cytogenetic diagnostics (both financially and technologically), the combination of different complementary molecular and molecular-cytogenetic techniques enables precise interpretation of genetic results. The choice of the screening method for subtelomere investigations largely depends also on the number of patients to be screened and on laboratory infrastructure.

Acknowledgments

This work was supported by the Ministry of High Education, Science, and Technology contract number RP L3-2145. The authors wish to thank Dr Mariano Rocchi for the locus-specific FISH probes and Dr Dianne Jones for English language editing assistance.

References

1 Flint J, Wilkie AO, Buckle VJ, Winter RM, Holland AJ, McDermid HE. The detection of subtelomeric chromosomal rearrangements in idiopathic mental retardation. Nat Genet. 1995;9:132-40. Medline:7719339

2 Saccone S, De Sario A, Della Valle G, Bernardi G. The highest gene concentrations in the human genome are in telomeric bands of metaphase chromosomes. Proc Natl Acad Sci U S A. 1992;89:4913-7. Medline:1594593

3 A complete set of human telomeric probes and their clinical application. National Institutes of Health and Institute of Molecular Medicine collaboration. Nat Genet. 1996;14:86-9. Medline:8782825

4 Knight SJ, Lese CM, Precht KS, Kuc J, Ning Y, Lucas S, et al. An optimized set of human telomere clones for studying telomere integrity and architecture. Am J Hum Genet. 2000;67:320-32. Medline:10869233

5 Knight SJ, Horsley SW, Regan R, Lawrie NM, Maher EJ, Cardy DL, et al. Development and clinical application of an innovative fluorescence in situ hybridization technique which detects submicroscopic rearrangements involving telomeres. Eur J Hum Genet. 1997;5:1-8. Medline:9156314

6 Fan YS, Siu VM, Jung JH, Xu J. Sensitivity of multiple color spectral karyotyping in detecting small interchromosomal rearrangements. Genet Test. 2000;4:9-14. Medline:10794355

7 Uhrig S, Schuffenhauer S, Fauth C, Wirtz A, Daumer-Haas C, Apacik C, et al. Multiplex-FISH for pre- and postnatal diagnostic applications. Am J Hum Genet. 1999;65:448-62. Medline:10417288

8 Sismani C, Armour JA, Flint J, Girgalli C, Regan R, Patsalis PC. Screening for subtelomeric chromosome abnormalities in children with idiopathic mental retardation using multiprobe telomeric FISH and the new MAPH telomeric assay. Eur J Hum Genet. 2001;9:527-32. Medline:11464244

9 Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. Nucleic Acids Res. 2002;30:e57. Medline:12060695

10 Kirchhoff M, Rose H, Lundsteen C. Deletions below 10 megabase pairs are detected in comparative genomic hybridization by standard reference intervals. Genes Chromosomes Cancer. 1999;25:410-3. Medline:10398439

11 Kirchhoff M, Gerdes T, Maahr J, Rose H, Bentz M, Dohner H, et al. Deletions below 10 megabasepairs are detected in comparative genomic hybridization by standard reference intervals. J Med Genet. 2001;38:740-4. Medline:11694545

12 Solinas-Toldo S, Lampel S, Stilgenbauer S, Nickolenko J, Benner A, Dohner H, et al. Matrix-based comparative genomic hybridization: biochips to screen for genomic imbalances. Genes Chromosomes Cancer. 1997;20:399-407. Medline:9408757

13 Shaw-Smith C, Redon R, Rickman L, Rio M, Willatt L, Fiegler H, et al. Microarray based comparative genomic hybridisation (array-comparative genomic hybridization) detects submicroscopic chromosomal deletions and duplications in patients with learning disability/mental retardation and dysmorphic features. J Med Genet. 2004;41:241-8. Medline:15060094

14 Vissers LE, de Vries BB, Osoegawa K, Janssen IM, Feuth T, Choy CO, et al. Array-based comparative genomic hybridization for the genomewide detection of submicroscopic chromosomal abnormalities. Am J Hum Genet. 2003;73:1261-70. Medline:14628292

15 Bonifacio S, Centrone C, Da Prato L, Scordo MR, Estienne M, Torricelli F. Use of primed in situ labeling (PRINS) for the detection of telomeric deletions associated with mental retardation. Cytogenet Cell Genet. 2001;93:16-8. Medline:11474170

16 Flint J, Knight S. The use of telomere probes to investigate submicroscopic rearrangements associated with mental retardation. Curr Opin Genet Dev. 2003;13:310-6. Medline:12787795

17 Dutrillaux B, Couturier J. The practical guide to chromosome analysis [in French]. Paris: Masson; 1981.

18 Rooms L, Reyniers E, van Luijk R, Scheers S, Wauters J, Ceulemans B, et al. Subtelomeric deletions detected in patients with idiopathic mental retardation using multiplex ligation-dependent probe amplification (MLPA). Hum Mutat. 2004;23:17-21. Medline:14695528

19 Kallioniemi A, Kallioniemi OP, Sudar D, Rutovitz D, Gray JW, Waldman F, et al. Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science. 1992;258:818-21. Medline:1359641

20 Kokalj-Vokac N, Marcun-Varda N, Zagorac A, Erjavec-Skerget A, Zagradisnik B, Todorovic M, et al. Subterminal deletion/duplication event in an affected male due to maternal X chromosome pericentric inversion. Eur J Pediatr. 2004;163:658-63. Medline:15309625

21 Varda NM, Kokalj Vokac N, Kanic Z, Bracic K, Erjavec A, Zagradisnik B, et al. Early renal insufficiency in a neonate with de novo partial trisomy of chromosome 10q. Am J Med Genet A. 2003;123:164-8. Medline:14598340

22 Bocian E, Helias-Rodzewicz Z, Suchenek K, Obersztyn E, Kutkowska-Kazmierczak A, Stankiewicz P, et al. Subtelomeric rearrangements: results from FISH studies in 84 families with idiopathic mental retardation. Med Sci Monit. 2004;10:CR143-51. Medline:15039644

23 Ravnan JB, Tepperberg JH, Papenhausen P, Lamb AN, Hedrick J, Eash D, et al. Subtelomere FISH analysis of 11688 cases: an evaluation of the frequency and pattern of subtelomere rearrangements in individuals with developmental disabilities. J Med Genet. 2006;43:478-89. Medline:16199540

24 Macina RA, Negorev DG, Spais C, Ruthig LA, Hu XL, Riethman HC. Sequence organization of the human chromosome 2q telomere. Hum Mol Genet. 1994;3:1847-53. Medline:7545974

25 Anderlid BM, Schoumans J, Anneren G, Sahlen S, Kyllerman M, Vujic M, et al. Subtelomeric rearrangements detected in patients with idiopathic mental retardation. Am J Med Genet. 2002;107:275-84. Medline:11840483

26 Ballif BC, Kashork CD, Shaffer LG. The promise and pitfalls of telomere region-specific probes. Am J Hum Genet. 2000;67:1356-9. Medline:11032793

27 Knight SJ, Flint J. Perfect endings: a review of subtelomeric probes and their use in clinical diagnosis. J Med Genet. 2000;37:401-9. Medline:10851249

28 Koolen DA, Nillesen WM, Versteeg MH, Merkx GF, Knoers NV, Kets MJ, et al. Screening for subtelomeric rearrangements in 210 patients with unexplained mental retardation using multiplex ligation dependent probe amplification (MLPA). J Med Genet. 2004;41:892-9. Medline:15591274

29 Rooms L, Reyniers E, Kooy RF. Subtelomeric rearrangements in the mentally retarded: a comparison of detection methods. Hum Mutat. 2005;25:513-24. Medline:15880643

30 Schoumans J, Nielsen K, Jeppesen I, Anderlid BM, Blennow E, Brondum-Nielsen K, et al. A comparison of different metaphase CGH methods for the detection of cryptic chromosome aberrations of defined size. Eur J Hum Genet. 2004;12:447-54. Medline:15026784

31 Joly G, Lapierre JM, Ozilou C, Gosset P, Aurias A, de Blois MC, et al. Comparative genomic hybridisation in mentally retarded patients with dysmorphic features and a normal karyotype. Clin Genet. 2001;60:212-9. Medline:11595023

32 Kirchhoff M, Pedersen S, Kjeldsen E, Rose H, Duno M, Kolvraa S, et al. Prospective study comparing HR-CGH and subtelomeric FISH for investigation of individuals with mental retardation and dysmorphic features and an update of a study using only HR-CGH. Am J Med Genet A. 2004;127:111-7. Medline:15108196

33 de Vries BB, Pfundt R, Leisink M, Koolen DA, Vissers LE, Janssen IM, et al. Diagnostic genome profiling in mental retardation. Am J Hum Genet. 2005;77:606-16. Medline:16175506

34 Schoumans J, Ruivenkamp C, Holmberg E, Kyllerman M, Anderlid BM, Nordenskjold M. Detection of chromosomal imbalances in children with idiopathic mental retardation by array based comparative genomic hybridisation (array-CGH). J Med Genet. 2005;42:699-705. Medline:16141005

35 Palomares M, Delicado A, Lapunzina P, Arjona D, Aminoso C, Arcas J, et al. MLPA vs multiprobe FISH: comparison of two methods for the screening of subtelomeric rearrangements in 50 patients with idiopathic mental retardation. Clin Genet. 2006;69:228-33. Medline:16542387