Subtelomeric Rearrangements Detected in Patients With Idiopathic Mental Retardation

Britt-Marie Anderlid,1* Jacqueline Schoumans,1 Göran Annerén,2 Sigrid Sahlén,1 Mårten Kyllerman,3 Mihailo Vujic,4 Bengt Hagberg,3 Elisabeth Blennow,1 and Magnus Nordenskjöld1

1Department of Molecular Medicine, Clinical Genetic Unit, Karolinska Hospital, Stockholm, Sweden
2Department of Clinical Genetics, Uppsala University Children's Hospital, Sweden
3Queen Silvia Children's Hospital, Section of Neuropediatrics, Göteborg University, Sweden
4Department of Clinical Genetics, Sahlgrenska University Hospital/Ostra, Göteborg, Sweden

A screening for submicroscopic rearrangements was performed in 111 patients with idiopathic mental retardation (MR) using fluorescence in situ hybridization (FISH) probes from the subtelomeric regions of all chromosome arms. Ten cryptic rearrangements were found (9%): five de novo deletions; one unbalanced de novo translocation; three unbalanced inherited translocations; and one unbalanced recombinant chromosome, inherited from a parent with a pericentric inversion. In addition, 50 of the patients were screened for interstitial rearrangements with spectral karyotyping (SKY), but no aberrations were found. However, SKY detected the subtelomeric rearrangement in three of the four unbalanced translocations. Dysmorphic features were present in all patients with detected subtelomeric rearrangements. © 2001 Wiley-Liss, Inc.

KEY WORDS: mental retardation; FISH; SKY; subtelomeric; cryptic chromosomal aberration; chromosome deletion; chromosome translocation

INTRODUCTION

Mental retardation (MR) is a common disorder and affects 1–3% of the population, yet the pathogenesis is poorly understood. The prevalence of mild MR (IQ 50–70) varies between study populations, while the prevalence of moderate–severe MR (IQ below 50) is between 0.3% and 0.5% in most studies [Roeleveld et al., 1997]. The etiology remains unknown in about half of the cases, despite thorough clinical and laboratory investigations [Flint and Wilkie, 1996]. However, several lines of evidence indicate that genetic factors are involved in many of the idiopathic cases, as they often show prenatal signs such as dysmorphic features, growth retardation, and malformations, or have a family history of MR.

Most autosomal chromosomal rearrangements that alter the gene dosage result in MR. Unbalanced chromosomal translocations with deletions of chromosome ends or truncation of the chromosome end followed by stabilization through addition of telomeric repeats is the cause of MR in several syndromes, such as the Wolf-Hirschhorn, cri du chat, and Miller-Dieker syndromes [Ledbetter, 1992]. Large aberrations are detectable with standard chromosome analysis, but since the resolution of this method is 3–5 Mb, smaller rearrangements have to be detected by other methods.

The subtelomeric regions are interesting from a genomic perspective, as they are gene-rich and often involved in chromosomal rearrangements [Saccone et al., 1992]. Most telomers stain light with G-banding, and small rearrangements are therefore difficult to detect. In the first study to screen individuals with idiopathic MR using subtelomeric polymorphic DNA markers, it was estimated that subtelomeric rearrangements could be the cause in 6% of the 99 cases [Flint et al., 1995]. 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 recently announced [National Institutes of Health, 1996; Knight et al., 2000]. These probes made it possible to analyze all chromosome ends for subtelomeric rearrangements. Subtelomeric screening with FISH was performed in 284 patients with moderate to severe MR, and in 182 patients with mild MR. Subtelomeric rearrangements
were found in 21 of the patients with moderate to severe MR (7.4%), and in one patient with mild MR (0.5%) [Knight et al., 1999]. The methods used have been time-consuming and expensive, and therefore a general screening of all children with idiopathic MR has been impossible. Accordingly, there is a need for clinical screening of all children with idiopathic MR (7.4%), and in one patient with mild MR (0.5%) were found in 21 of the patients with moderate to severe MR. They had all been extensively evaluated, and standard chromosome analysis (approximately 400–450 band resolution) had been classified as normal in all. Seventeen patients had an Angelman or Angelman-like phenotype with negative DNA analysis. *FMR1* mutation was excluded in patients with a phenotype suggestive of fragile X syndrome. In the other cases, no apparent syndrome was recognizable. Pediatric neurologists referred 75 patients, and 36 patients were recruited from clinical genetics centers. The age-span of the group was 1–51 years on investigation. Only four patients were older than 20 years, and the mean age was 9.3 years. All cases were characterized by the following five criteria: level of MR (mild = IQ of 50–70; moderate to severe = IQ below 50); presence of behavioral disorder (autistic symptoms or autism, Asperger syndrome, hyperactivity, ADHD, or sleeping disturbance); dysmorphic features (facial anomalies, abnormalities of hands, feet, or body constitution); malformations (brain, internal, or genital); and family history of MR. Moderate or severe MR was present in 82 cases (46 males and 36 females), and mild MR in 29 (10 males and 19 females). A behavioral disorder was diagnosed in 56 of the patients (50%), dysmorphic features in 77 (69%), malformation in 26 (23%), and 40 of the cases had a family history of MR (36%). In the 29 cases with mild MR, all had one or several of the criteria for dysmorphic features, malformation, and family history.

**Patient 1**

This girl, born in 1988, was the oldest of three siblings. She was born in gestational week 33 and had a BW of 2,040 g (+0.5 SD). The patient had mild MR with delayed motor function and was in need of special education. She was tall and had dysmorphic features including protruding ears, pes planus, thin ankles, and prominent lumbar lordosis. The parents were unrelated. Her younger brother was healthy but her sister, born in 1992, had mild MR, macrocephaly, and short stature. No other cases of MR were known in the family.

**Patient 2**

A girl born in 1999, who was the third child born to unrelated healthy parents. She had severe MR, severe delay in motor function, an atrial septal defect, a hoarse cry, and dysmorphic craniofacial features with a broad nasal bridge, hypertelorism, and brachycephaly. Her BW and postnatal growth were normal. The father had two paternal cousins who had experienced late intrauterine fetal deaths, and one paternal cousin with “cerebral palsy” and MR.

**Patient 3**

A girl born in 1996, as the second child of healthy nonconsanguineous parents. The older sister was healthy, and a healthy brother had recently been born. Patient 3 was born in gestational week 37 and had a BW of 2,460 g (−1 SD) and a BL of 46 cm (−1 SD). At the age of 3 1/2 years she could walk with support but had no language. She had severe MR, short stature (−3 SD), epilepsy, and dysmorphic features with hypertelorism, downward slant of palpebral fissures, broad forehead, and low-set ears. Magnetic resonance imaging (MRI) of the brain was performed at 2 years of age and showed diminished white substance.

**Patient 4**

A boy born in 1995, who was the youngest of three children born to healthy, unrelated parents. Patient 4 was born in gestational week 40 with a BW of 3,240 g (−0.5 SD), BL of 44 cm (−3.5 SD), and OFC of 34.2 cm. He had severe MR, hyperactivity, sleeping disturbance, microcephaly, bilateral syndactyly of the second and third toes, inguinal hernia, skeletal malformations (vertebras and ribs), and epilepsy. This patient had facial anomalies including a high, broad nasal bridge; temporal narrowing; long eyelashes; large mouth with tented upper lip; and large ears. The older sister was healthy, but the older brother, born in 1990, had severe MR and a clinical picture resembling Angelman syndrome. DNA testing for Angelman syndrome was negative in both brothers. Two maternal male cousins of the boys had MR.

**Patients 5a and b**

These siblings, a boy born in 1990 and a girl born in 1995, were the only children of healthy, nonconsanguineous parents. Both siblings were of normal size at birth. The children had similar dysmorphic facial characteristics with a hypoplastic middle face, high forehead, short pointed nose, narrow palpebral fissures, asymmetrically-sized eye globes, thin upper lip, and a high palate. The boy had severe MR and autism, while the sister had severe MR together with milder autistic features. Both children had wide supratentorial
ventricles and heterotopias determined by MRI. In addition, the boy had a thin corpus callosum. They both had severe feeding difficulties leading to malnutrition and growth retardation (−2 SD). A paternal uncle was mentally retarded and died of a cerebral hemorrhage as an adult.

Patient 6

This woman, born in 1977, was the younger of two sisters born to unrelated parents. Her BW was 2,700 g in gestational week 41 (−1.8 SD), she started to walk when she was 3 years old, and she had a severe delay in speech development. The woman had severe MR, an autistic disturbance, and epilepsy combined with synophrys, hypertelorism, and strabismus. The sister and the parents were healthy and there was no family history of MR.

Patient 7

This boy, born in 1984, was the oldest of three children born to nonconsanguineous parents. He had severe MR, and communicated with sign language. He was of normal size at birth but developed postnatal growth retardation (−4 SD) and had low levels of growth hormone. The boy had an Arnold Chiari type 1 malformation, mitral valve insufficiency, bilateral undescended testes, severe myopia, frontal bossing, low-set and malformed ears, narrow face, deep-set eyes, a thin nose with a high bridge, down-turned corners of the mouth, receding chin, and slender hands and feet. A maternal and a paternal cousin had MR, but they shared no other features with patient 7.

Patient 8

A girl born in 1982, the second child of healthy, unrelated parents. She had mild MR, pre- and postnatal growth retardation, ataxia, autistic features, cleft palate, ventricular septal defect, congenital nystagmus, and facial anomalies. MRI of the brain showed partial agenesis of the corpus callosus. She had two healthy brothers, but a paternal half sister, a half brother of the father, and a nephew of the father all had MR and different malformations.

Patient 9

A girl, born in 1997 as the first child of healthy, unrelated parents. She was born in gestational week 34 and had a BW of 2,270 g (+0.5 SD). She had severe MR, lack in emotional contact, and breath-holding spells. She had low-set ears, upslanted palpebral fissures, epicanthal folds, flat base of the nose, thin philtrum, hypoplastic middle face, and bilateral single transverse creases. MRI of the brain at 20 months of age showed a thin corpus callosum and pathological changes in the white substance.

Patient 10

This woman, born in 1968, was the youngest of three siblings. Her BW and growth was normal, and in infancy she had delayed speech development. This patient had mild MR, an autistic disturbance, epilepsy, and ataxic gait. She was slender, with thin shoulders and long fingers and toes, and had discrete facial findings with a long face and high nasal bridge. During the last years there had been a decline in speech functions and activity of daily living abilities. The parents were not related and there was no family history of MR, but she had a paternal cousin with cerebral palsy and ataxia.

Cytogenetic Studies

Metaphase slides were prepared from lymphocyte cultures of peripheral blood. Chromosome analysis was performed according to routine procedures using GTG banding.

FISH

The Chromoprobe Multiprobe T System (Cytocell Ltd., Adderbury, England) was used to screen the subtelomeric regions [Knight et al., 1997]. The test included 41 subtelomeric probes (p-arms from the acrocentric chromosome ends not included), and the device made it possible to analyze all 41 chromosome ends on one slide. The probes in this commercial kit were changed during the course of this study, from initially mostly cosmids (first-generation subtelomeric probes) to mostly PAC probes (second-generation probes) [National Institutes of Health, 1996; Knight et al., 2000]. Thirty-seven of the patients were analyzed with the new set of probes. Probes from the p-arms were labeled with FITC and q-arms with Cy3 (first generation) or Texas Red (second generation), indirectly labeled in the first-generation kit and directly labeled in the second. The manufacturer's protocol was used with minor modifications. Metaphase slides were pretreated with 2 × SSC at 37°C for 30 min followed by pepsin (0.1 mg/ml in 0.01 M HCl) at 37°C for 2–4 min and denatured at 75°C for 2 min. After hybridization overnight in a 37°C incubator or HYBrite denaturation/hybridization system (Vysis, Downers Grove, IL), signals were amplified through three steps of fluorescently-labeled antibodies (first generation) before counterstaining with DAPI, or slides were counterstained directly after washing (second generation). The slides were analyzed in a Zeiss Axioskop II fluorescence microscope (Zeiss, Göttingen, Germany) and images were captured by a cooled CCD camera (Sensys Photometrix, München, Germany) and the software Smart Capture (Digital Scientific, Cambridge, UK), using a Power Macintosh computer. Inverted DAPI staining was used for chromosome identification during FISH analysis.

A minimum of four metaphases were examined for each chromosome. Positive results were confirmed with a separate FISH analysis with the same probes as used in the Multiprobe T device and probes from the second-generation set. In addition, the TelVysion subtelomeric probes (Vysis) were used in cases in which a deletion was detected.
SKY

The SKY method was described by Schröck et al. [1996]. The SKY probe mixture and hybridization reagents were prepared by Applied Spectral Imaging (Migdal HaEmek, Israel). Combinatorial labeling of five fluorochromes was used to generate the 24 colors. Hybridization and detection were carried out according to the protocol provided with the Sky Paint™ hybridization and detection kit (Applied Spectral Imaging, Migdal Ha Emek, Israel). The slides were incubated for 48 hr at 37°C in a humidified chamber. After washing and amplification according to the manufacturer's protocol, the slides were counterstained with DAPI. Metaphases were captured using the SD200 spectral imaging system Spectral Cube (Applied Spectral Imaging), connected to a Zeiss Axioscope II fluorescence microscope with triple bandpass optical filter (Croma Technology, Brattleboro, VT). DAPI images were acquired using a DAPI filter. In each case a minimum of four metaphases were analyzed using the SKY view software (Applied Spectral Imaging).

RESULTS

A total of 111 patients were analyzed with FISH, using subtelomeric probes. In 10 cases an aberration of the subtelomeric regions was discovered. De novo deletions were found in five patients and unbalanced translocations in four. One of the unbalanced translocations was de novo and three cases were inherited from balanced carrier parents. In two siblings with an unbalanced recombinant chromosome, the father carried a large pericentric inversion. The results and a summary of the phenotypic characteristics of the cases with aberrations are compiled in Table I.

All cases with subtelomeric rearrangements were reexamined with high-resolution (minimum 550 bands) chromosome analysis. In retrospect, the derivative chromosomes could be identified as aberrant in cases 2, 5a and b, and 7 (Fig. 1a,b), and in cases 2 and 7 the aberrations were also detectable with standard resolution. In all other cases, the aberration could not be confirmed by high-resolution karyotyping.

Cross-hybridization between chromosomes was frequently observed with the Multiprobe T device, and is caused by polymorphic subtelomeric repeats shared by several chromosome ends. For the first set of probes the most common cross-hybridizations were 11p–17p, 12p–6p and/or 20q and 9q–18p, and for the second-generation probes 8p–1p, 9q–18p, 11p–17p, 15q–interstitial 1q and/or interstitial 15q, 17p–interstitial 17p, 17q–1p and/or 6q and 22q–interstitial 2p.

The four cases with unbalanced translocations, including their parents, were investigated with SKY to evaluate the sensitivity of this method in searching for subtelomeric rearrangements, and to make a rough estimation of the size of the rearrangement. The translocation in patient 8 was not detectable, but the other three translocations, both in unbalanced and balanced form, were easily confirmed by SKY (Fig. 1a,b).

| Table 1: Results and Characterization of the Ten Cases Where Aberrations Were Found |

<table>
<thead>
<tr>
<th>Case</th>
<th>Year of birth</th>
<th>Gender</th>
<th>MR</th>
<th>Behavioral disorder</th>
<th>Dysmorphology</th>
<th>Malformation of MR</th>
<th>Subtelomere FISH</th>
<th>SKY</th>
<th>G-band</th>
<th>Parents</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1988</td>
<td>Female</td>
<td>Mild</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>1999</td>
<td>Female</td>
<td>Severe</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Pos</td>
</tr>
<tr>
<td>3</td>
<td>1996</td>
<td>Female</td>
<td>Severe</td>
<td>Yes</td>
<td>No</td>
<td>Postnatal</td>
<td>No</td>
<td>No</td>
<td>Pos</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>1995</td>
<td>Male</td>
<td>Severe</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Prenatal</td>
<td>Yes</td>
<td>No</td>
<td>Pos</td>
</tr>
<tr>
<td>5a</td>
<td>1990</td>
<td>Male</td>
<td>Severe</td>
<td>Yes</td>
<td>Autistic features</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>5b</td>
<td>1995</td>
<td>Female</td>
<td>Severe</td>
<td>Yes</td>
<td>Autistic features</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>1977</td>
<td>Female</td>
<td>Mild</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>1997</td>
<td>Female</td>
<td>Severe</td>
<td>Yes</td>
<td>No</td>
<td>Postnatal</td>
<td>Yes</td>
<td>Yes</td>
<td>Neg</td>
<td>Neg</td>
</tr>
<tr>
<td>8</td>
<td>1997</td>
<td>Female</td>
<td>Severe</td>
<td>Yes</td>
<td>Autonomous features</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>9</td>
<td>1997</td>
<td>Female</td>
<td>Mild</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>10</td>
<td>1998</td>
<td>Female</td>
<td>Mild</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

* Positive SKY and G-banding indicates that the aberration is detectable with SKY and high resolution G-banding, respectively.

* Cases with discordant phenotype including MR but no detectable chromosomal aberration.
Fig. 1a. FISH analysis with the subtelomeric probes detecting rearrangements on metaphases from patients 2, 3, 5. P-arm probes are labeled in green and q-arm probes in red. Arrows point at abnormal subtelomere signals. Insets show SKY-paint (RGB picture and classified picture) and G-banded chromosomes.
Fig. 1b. FISH analysis with the subtelomeric probes detecting rearrangements on metaphases from patients 8 and 9 and the mother of patient 7. P-arm probes are labeled in green and q-arm probes in red. Arrows point at abnormal signals. Insets show SKY-paint and G-banded chromosomes.
Fifty of the patients, negative in the subtelomeric screening, were also investigated with SKY to search for interstitial rearrangements, but no additional aberrations could be detected.

**Patient 1**

The subtelomeric screening revealed a deletion of 2q (cosmid 210E14), 46,XX.del(2)(qter), which could not be detected on high-resolution chromosome analysis (not shown). FISH analysis with a commercial probe (TelVysion 2q, Vysis) from subtelomeric 2q confirmed the deletion. No aberration was found in the sister, who had mild MR and dysmorphic features, and both parents had normal signals from 2q (TelVysion 2q, Vysis).

**Patient 2**

The subtelomeric screening disclosed an unbalanced translocation with trisomy of terminal 2q and monosomy of terminal 4q, 46,XX.der(4)t(2;4)(qter;qter) (Fig. 1a). The derivative chromosome could be identified on standard resolution chromosome analysis (450 band resolution) in retrospect and with SKY. Analysis of the parents revealed a balanced translocation, 46,XY.t(2;4)(qter;qter), in the father Fig. 1a, who in turn had inherited the translocation from his father. Further family analysis is ongoing.

**Patient 3**

Screening with subtelomeric probes showed a deletion of 4p, 46,XX.del(4)(qter), which was confirmed with two different subtelomeric probes (PAC-probe 36P21 and the TelVysion 4p, Vysis) and a probe from the Wolf-Hirschhorn critical region (WHSCR) (Cytocell Ltd., Adderbury, England). The deletion could not be detected on high-resolution chromosome analysis (Fig. 1a). No aberration involving 4p could be found in the parents.

**Patient 4**

During subtelomeric screening a deletion of terminal 6q, 46,XY.del(6)(qter), was discovered (not shown). The deletion was confirmed with another subtelomeric probe (TelVysion 6q, Vysis), and using this probe, two normal signals were present in both the brother and the parents. The deletion was thus de novo in the patient, indicating that this is not a polymorphism. It was not possible to confirm the deletion on high-resolution chromosome analysis.

**Patients 5a and b**

The subtelomeric screening revealed a loss of one signal from 6q and an extra signal from 6p on 6qter, indicating monosomy of 6qter and trisomy of 6pter (Fig. 1a). G-banded high-resolution chromosome analysis (600 bands) could detect an extra band on terminal 6q. Analysis of the parents showed a large, pericentric inversion of chromosome 6 in the father, 46,XY.inv(6)(p23q27), which could be detected by standard chromosome banding (400 bands). Thus, the karyotypes of the siblings were 46,XY.rec(6)dup(6p)inv(6)(p23q27) and 46,XX.rec(6)dup(6p)inv(6)(p23q27).

**Patient 6**

The subtelomeric screening disclosed a deletion of 9q, 46,XX.del(9)(qter) (not shown), which could not be detected on reevaluation of the G-banded high-resolution chromosomes. The deletion was confirmed with a subtelomeric 9q probe (TelVysion 9q, Vysis). Both parents had normal FISH results when analyzed with the 9q-probe.

**Patient 7**

The screening showed an unbalanced translocation with trisomy of terminal 9q and monosomy of terminal 21q, 46,XY,der(21)t(9;21)(qter;qter) (not shown). Analysis of the parents revealed that the mother was a carrier of a balanced translocation, 46,XX,t(9;21)(qter;qter) (Fig. 1b), and further family investigations showed this translocation to be de novo in the mother. The derivative chromosome 21 could be identified on the 400 band level, in both the boy and the mother, and the balanced translocation could be detected by SKY (Fig. 1b).

**Patient 8**

Subtelomeric screening revealed an unbalanced translocation with trisomy of terminal 12q and monosomy of terminal 17q, 46,XX.der(17)t(12;17)(qter;qter) (Fig. 1b). This rearrangement could not be detected with high-resolution chromosome analysis or SKY. The father carries a balanced translocation, 46,XY,t(12;17)(qter;qter). Further family investigations are ongoing.

**Patient 9**

The screening of subtelomeric regions revealed an unbalanced translocation with monosomy of terminal 22q and trisomy of terminal 20q, 46,XX,der(20)t(20;22)(qter;qter) (Fig. 1b). FISH analysis with the 20q and 22q probes was normal in both parents. Reevaluation of G-banded high-resolution chromosomes could not detect the rearrangement, but the unbalanced translocation could be identified by SKY. The parents chose to undergo prenatal testing for the detected rearrangement during an ongoing pregnancy, and the result was normal as expected.

**Patient 10**

The subtelomeric screening showed a deletion of terminal 22q, 46,XX.del(22)(qter) (not shown). The result was confirmed with repeated analyses with the cosmid probe (N85A3). Additional testing with two different probes (PAC probe 99K24 and TelVysion 22q, Vysis) showed signals from both chromosomes 22 with a minor difference in signal strength, indicating a possible breakpoint. FISH analysis with a probe from the ARSA gene was normal. The mother and both
siblings had normal results on FISH analysis with both the cosmid and the PAC probe. The father was deceased and therefore not available for testing. The deletion could not be detected on G-banded high-resolution chromosome analysis.

In this series of patients the overall prevalence of subtelomeric rearrangements was 9% (10/111). In the group with moderate to severe MR, an aberration was found in 8.5% (7/82), and three cases of 29 (10.3%) with mild MR were found to have a subtelomeric rearrangement. Of the 111 patients analyzed, 77 had dysmorphic features, and all cases with subtelomeric rearrangements were found in this group ($\chi^2 = 4.94, P < 0.05$). A behavioral disorder was described in five of the cases with subtelomeric rearrangement (compared to 56 of the 111 in the study group), and malformations were present in four (compared to 26 of 111). Six patients had pre- or postnatal growth retardation (data from all included patients not known). A family history of MR was present in six cases (compared to 40 of 111), but only in four of them was the aberration inherited. The male : female ratio of the cases in which an abnormality was found was 3:7, compared to the 1:1 ratio in the study group. The number of positive cases in relation to phenotype is summarized in Table II. The detection rate increased if patients with both dysmorphic features and family history of MR were selected. In the 12 patients with severe MR, dysmorphic features, and family history, a subtelomeric rearrangement was found in three. In three of the 10 positive cases, the aberration could be detected by chromosome analysis, either by high-resolution banding (patients 5a and b) or on reexamination of standard banding (patients 2 and 7).

**DISCUSSION**

We detected subtelomeric chromosome abnormalities in 10 cases with idiopathic MR in a screening of 111 patients. In several cases a genotype–phenotype correlation could be made. Patient 3, with a subtelomeric deletion of 4p, has several of the clinical signs of Wolf-Hirschhorn syndrome (WHS), including the characteristic facial features, postnatal growth retardation, MR, and severe MR [Zollino et al., 2000]. However, this patient was not diagnosed with WHS prior to the chromosome finding. In patients 5a and b the phenotype corresponds well to earlier described cases with duplication of 6p and deletion of 6q. Wauters et al. [1993] described two patients with recombinant chromosomes 6 with breakpoints very similar to our cases (trisomy 6p23-pter and monosomy 6q27-qter). Their phenotype included failure to thrive, pre- and postnatal growth retardation, prominent forehead, micro-ophthalmia, blepharophimosis, abnormal and low-set ears, short and pointed nose, thin lips, and dilated lateral ventricles [Wauters et al., 1993; Röthlisberger et al., 1999]. The phenotype in patient 7 includes several features reported in cases with trisomy 9qter, such as growth retardation, MR, congenital heart disease, prominent nasal bridge, deep-set eyes, and a small, receding mandible [Turleau et al., 1975; Spinner et al., 1993]. Patient 10 probably carries a very small deletion of 22qter, but the clinical picture is consistent with other reports of related deletions, including MR, normal pre- and postnatal growth, hypotonia, speech delay, epicantal folds, and abnormal nose [Nesslinger et al., 1994]. One case with a 60 kb deletion of subtelomeric 22q and mild phenotype has been described [Flint et al., 1995], and there is one report of a 22qter deletion in combination with autism [Goizet et al., 2000].

In patient 8, the imbalances were not detectable with SKY or high-resolution chromosome analysis, suggesting a <1–2 Mb size for the fragments involved. However, an exact size determination demands extensive analysis with additional probes from the region. The family history in this case, with affected half siblings and cousins connected through the carrier father, strongly supports the etiological importance of the unbalanced translocation. The different phenotypes in the affected relatives could be explained by the inheritance of different derivative chromosomes leading to different chromosomal imbalances.

Patients 1, 4, and 6 have deletions of chromosomes 2qter, 6qter, and 9qter, respectively. In none of the cases was the aberration found in the parents, making it unlikely that the detected deletions were polymorphisms. Subtelomeric 2q harbors a polymorphic site, and the probes from this location show a polymorphic pattern [Macina et al., 1994]. Deletions have been found in healthy individuals, using both the cosmid and the PAC probe [Ballif et al., 2000; Knight and Flint, 2000]. We observed this polymorphism in two patients, and in both cases one healthy parent had the same hybridization pattern with absence of one signal. Another commercial subtelomeric 2q probe (TelVysion2q, Vysis) is located proximal to the probe used in the Multiprobe T System and this probe is not reported to be polymorphic. The two patients with a supposed polymorphism had a normal signal pattern with the TelVysion-probe (not shown), while patient 1 was deleted.

Patients 1 and 6 both have siblings with MR but with discordant phenotypes, and the siblings did not carry
the abnormality detected in the index cases. In these families, two different etiological mechanisms must be present. It is also possible that the patients with the subtelomeric deletions have, in addition to the symptoms caused by the deletions, features related to their siblings' diseases.

In patients 2 and 9 the imbalances could be detected by SKY and/or G-banding, indicating that the aberrations were larger than 2–3 Mb. Imbalances of this size are likely to give a phenotype including MR.

In earlier studies of the prevalence of subtelomeric aberrations in populations with idiopathic MR, variable detection rates have been found [Flint et al., 1995; Knight et al., 1999; Slavotinek et al., 1999; Rossi et al., 2001]. This variation is probably correlated to the size of the study population and the inclusion criteria. In a survey of 29 cases with known subtelomeric rearrangements, 83% had two or more facial dysmorphic features, 50% had a family history, and 37% had prenatal growth retardation [de Vries et al., 2001]. The most significant difference from a control group of patients with idiopathic MR and no subtelomeric abnormality was in growth retardation and family history, while the difference between the two groups concerning dysmorphic features was not statistically significant. Our 10 patients all had dysmorphic features (compared to 69% in the study population). Six of the 10 patients had a family history of MR (compared to 36% in the study population), but two had de novo deletions, and thus an additional etiological mechanism for MR must be present in these families. Four of 10 patients had a family history and were inherited. Only two of the 10 patients had low birth-weight or low birth-length, but three other cases developed postnatal growth retardation. Three of 29 analyzed cases with mild MR were found to have a subtelomeric aberration, which makes the detection rate in this group high (10.3%) compared to the results (0.5%) of Knight et al. [1999]. This could be explained by the size of the study group, but also by selection, as all the analyzed cases with mild MR in our study had dysmorphic features, malformations, or a family history of MR. Two of the three positive cases had both dysmorphic features and a family history.

In a majority of FISH analyses, cross-hybridizations were present. In most cases the true subtelomeric signal was easy to discriminate from a cross-hybridization because of the difference in signal strength and the typical chromosomal localization. A signal from a cross-hybridization could be misinterpreted as a duplication, but this would be revealed by analysis of the parents.

Nonhomologous chromosomes share common telomere-associated repeats, and these may result in mispairing during meiosis and facilitate terminal translocations [Brown et al., 1990; LedBetter, 1992]. Although rearrangements are more common in the telomeric region, interstitial unbalanced rearrangements also occur and give rise to MR syndromes, i.e., Williams, Prader-Willi, Angelman, and Smith-Magenis syndromes. Hence, there is a need for a full genome screening method for chromosome imbalance.

Bezrookove et al. [2000] performed 24-color FISH in 21 individuals with abnormal phenotypes and normal G-banding, and two unbalanced translocations were discovered. Uhrig et al. [1999] used multiplex-FISH (M-FISH) to screen for cryptic rearrangements in 20 patients with normal karyotype by G-banding and a phenotype suggestive for chromosomal syndrome, and two cases with an unbalanced translocation were found. All four rearrangements detected in these two studies were telomeric and would have been found using subtelomeric FISH. Twenty-four-color FISH also has the disadvantage of not detecting intrachromosomal rearrangements, such as small deletions or duplications. In our screening of 50 patients with SKY, no interstitial rearrangements were found, and interstitial translocations and duplications are thought to be uncommon. As in conventional G-banding, the resolution of M-FISH and SKY is dependent on chromosome condensation, as well as on the fluorochrome composition of the probes involved in a rearrangement. If the fluorochrome compositions for the participating chromosomes are similar, small structural abnormalities will be difficult to detect. However, multicolor FISH labels each chromosome in a specific color. If this technique could be applied to the subtelomeres, the analysis of the subtelomeric regions could be simplified, and a small number of metaphases would be sufficient for a complete analysis of all chromosome ends.

Genome-wide screening could be performed using polymorphic markers or single nucleotide polymorphisms (SNPs). Automation is possible today, but in order to reach a sufficient resolution one would need a dense set of markers with a high degree of heterozygosity. This method also requires DNA from both the patient and the parents [Rosenberg et al., 2000]. The new microarray techniques are promising for whole-genome screening purposes, since as many as 20,000–30,000 loci can be examined in a single experiment. Using a CGH approach with hybridization of genomic DNA to arrays with probes from human genes, gene-dose differences can be discovered [Pollack et al., 1999].

In our study, four out of 10 cases were inherited, which is comparable to the 10 of 22 in the survey by Knight et al. [1999]. In familial cases the detection of a chromosomal aberration is of great importance in offering genetic counseling and prenatal testing. The high incidence of inherited cases strongly supports the use of subtelomeric screening of children with idiopathic MR.

The quality of the commercial kits of subtelomeric probes has improved significantly during the course of this study. Until a cheaper and less labor-intense method for screening for subtle chromosome abnormalities emerges, we recommend screening for subtelomeric rearrangements with FISH in all children with idiopathic MR combined with dysmorphic features, prenatal or postnatal growth retardation, or family history of MR. It is hoped that the delineation of MR syndromes and the identification of chromosomal regions and genes involved in neurodevelopment will improve our understanding of the biology of cognitive functions and dysmorphism. This will result in better care of
patients and families with MR syndromes, and may form a basis for new treatment strategies.

ACKNOWLEDGMENTS

We thank the participating clinical doctors for referring patients and sharing clinical data, and Dr. J. Flint and his lab for generously sharing the subtelomeric probes.

REFERENCES


