

## Familial Mental Retardation Syndrome ATR-16 Due to an Inherited Cryptic Subtelomeric Translocation, t(3;16)(q29;p13.3)

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### Summary

In the search for genetic causes of mental retardation, we have studied a five-generation family that includes 10 individuals in generations IV and V who are affected with mild-to-moderate mental retardation and mild, nonspecific dysmorphic features. The disease is inherited in a seemingly autosomal dominant fashion with reduced penetrance. The pedigree is unusual because of (1) its size and (2) the fact that individuals with the disease appear only in the last two generations, which is suggestive of anticipation. Standard clinical and laboratory screening protocols and extended cytogenetic analysis, including the use of high-resolution karyotyping and multiplex FISH (M-FISH), could not reveal the cause of the mental retardation. Therefore, a whole-genome scan was performed, by linkage analysis, with microsatellite markers. The phenotype was linked to chromosome 16p13.3, and, unexpectedly, a deletion of a part of 16pter was demonstrated in patients, similar to the deletion observed in patients with ATR-16 syndrome. Subsequent FISH analysis demonstrated that patients inherited a duplication of terminal 3q in addition to the deletion of 16p. FISH analysis of obligate carriers revealed that a balanced translocation between the terminal parts of 16p and 3q segregated in this family. This case reinforces the role of cryptic (cytogenetically invisible) subtelomeric translocations in mental retardation, which is estimated by others to be implicated in 5%–10% of cases.

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### Introduction

Mental retardation occurs in 0.5%–1% of the total population (Curry et al. 1997) and may be due to a variety of genetic and environmental factors. Frequent genetic causes include chromosome aneuploidies, such as Down syndrome; monogenic disorders, such as fragile X syndrome; and small interstitial or subtelomeric chromosomal deletions. From a counselor's point of view, the search for the causes of mental retardation is often very difficult and frustrating. Laboratory evaluation of patients with mental retardation tends to be limited to standard karyotyping of an affected individual, molecular detection of the fragile X syndrome, neuroimaging, and metabolic testing (e.g., of plasma amino acids and urine organic acids) (Curry et al. 1997). Despite these efforts, in ~50% of cases the absence of specific clinical or laboratory findings leads to a designation of idiopathic mental retardation, and families are assigned recurrence risks based on either the population risk or the pedigree analysis, which is limited by both the family size and the accuracy of the family history.

In the search for other underlying molecular genetic defects of mental retardation, a five-generation family that includes 10 individuals with mild-to-moderate mental retardation and mild, nonspecific dysmorphic features was studied. In this family, the disease appeared to be inherited in an autosomal dominant fashion. Interestingly, individuals with the disease appeared only in generations IV and V, which is suggestive of anticipation. Standard laboratory evaluation did not reveal any abnormalities. In addition, since submicroscopic deletions are a frequent cause of mental retardation with mild dysmorphic features, high-resolution karyotyping and multiplex FISH (M-FISH) were performed, but no evidence of chromosomal aberrations could be detected.

Since the size of the family was large enough to allow a genetic linkage search (genome search), we decided to scan the genome in a first step, to clarify the molecular

etiology of the disease and the inheritance pattern. Linkage was found to the distal region of chromosome 16p, and the most telomeric markers were deleted in the patients. Subsequent FISH analysis led to the discovery of a cryptic balanced subtelomeric translocation, t(3;16)(q29;p13.3), segregating in this family. The phenotype of the patients could be explained by subtelomeric deletion of chromosome 16p, sometimes referred to as "ATR-16 syndrome."

## Subjects and Methods

### Family

A pedigree of the family is shown in figure 1. The nuclear family, consisting of IV-9, IV-10, V-5, and V-7, came to the Department of Medical Genetics in Munich for genetic counseling because of suspected X-linked mental retardation. A detailed family history revealed affected females and male-to-male transmission, thereby excluding an X-linked pattern of inheritance. All affected individuals were examined by one of three authors of the present report (E.H.-F., I.R., or P.K.). Clinical data are summarized in table 1. Informed consent was obtained from all family members prior to linkage analysis.

### Genotyping

Genomic DNA was extracted, by salt extraction, from whole blood. Microsatellite markers of the Cooperative Human Linkage Center (CHLC) fluorescein-labeled human screening set (version 6a; Genome Systems) were used to perform a genome search in the family. Multiplex reactions of five markers per dye and three different dyes per lane were analyzed on an ABI automated sequencer. After identification of the candidate region, additional chromosome 16p13.3 markers (tel-D16S521-HBA2-D16S3024-D16S3124-D16S3070-D16S3027-D16S423-D16S3030-D16S418-D16S3020-cen) and chromosome 3q29 markers (cen-D3S1601-D3S3669-D3S2748-D3S1305-D3S3550-tel) were chosen from the Génethon human linkage map (Dib et al. 1996). For each of the Génethon markers, one of the primers was end-labeled with  $\gamma$ [<sup>32</sup>P]-ATP, and the PCR products were separated on 6% denaturing acrylamide gels and were visualized by autoradiography.

### Linkage Analysis

Pairwise LOD scores between the mental retardation locus and each marker were calculated by means of the MLINK program of the linkage package, version 5.1 (Lathrop and Lalouel 1984). The linkage calculation was done exclusively for patients, obligate carriers, and their spouses. For linkage calculation, obligate carriers were considered patients, and an autosomal dominant inher-

itance with 100% penetrance was assumed. The frequency of the disease was estimated at .00001, and equal allele frequencies (1/no. of alleles) were used for each marker.

### FISH

FISH was performed on Epstein-Barr virus-immortalized lymphoblastoid cells from carriers and patients. Probes CosRT1 (from the Rubenstein-Taybi locus), PAC 27M3 (containing marker D16S3040), and cC1-2 and cCBFS1, both of which were located close to but distal from the TSC2 gene (Nellist et al. 1993), were labeled by nick translation and were hybridized according to standard protocols. A biotin-labeled probe was detected by an FITC-conjugated avidin-detection system; a dioxigenin-labeled probe by a rhodamin-conjugated antibody dioxigenin-detection system. Subtelomere probes (Ning et al. 1996) dJ11286B18 (3pter) and 196F4 (3qter) were directly labeled with Cy5, 119L16 (16pter) with avidin Cy3.5, and D3b1 (16qter), with FITC. Probes were hybridized on metaphase spreads, and gray-value images were captured and overlaid by the Leica QFISH software package (Leica Microsystems Imaging Solutions).

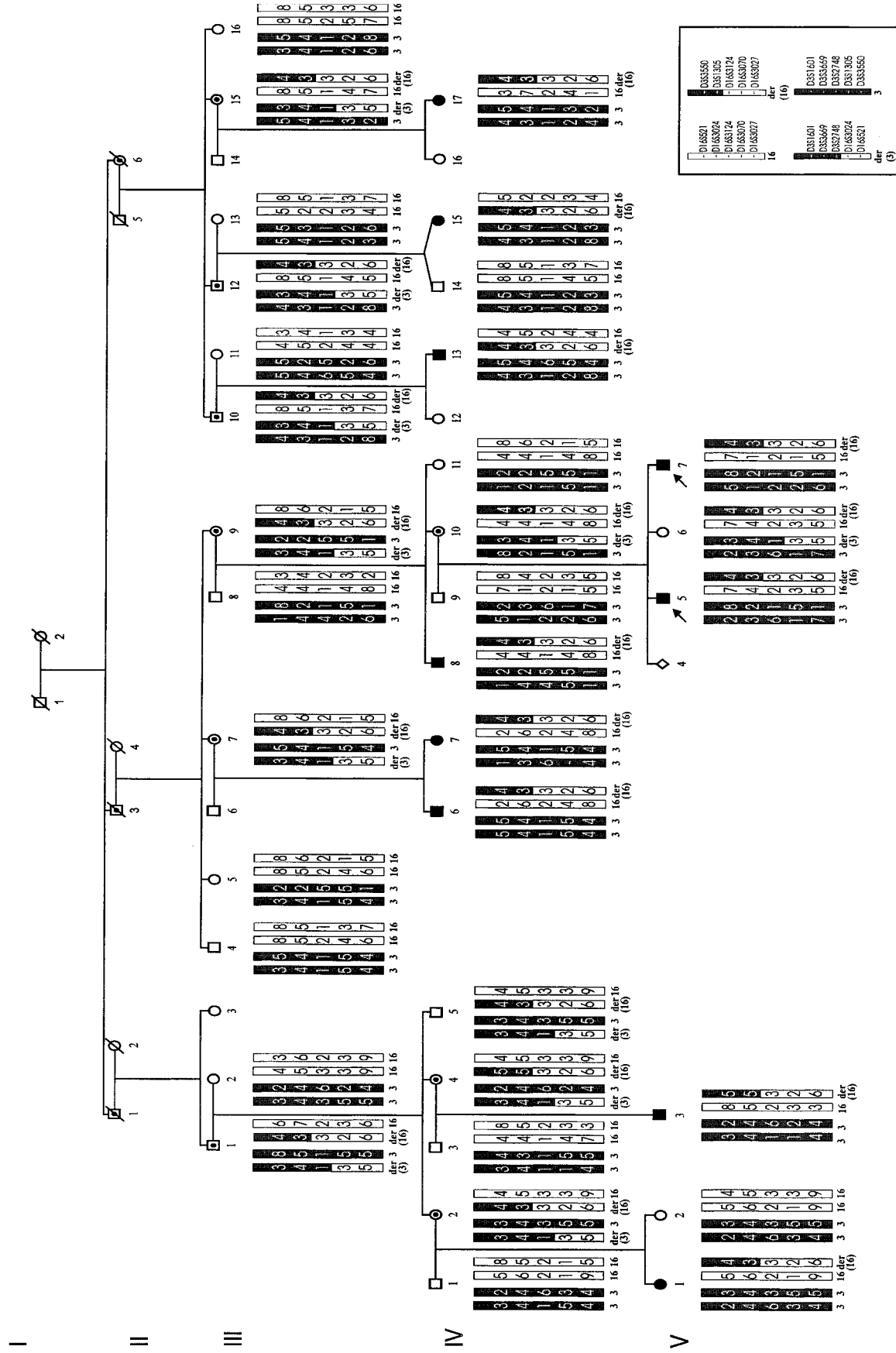
### M-FISH

M-FISH was performed as described elsewhere (Speicher et al. 1996; Eils et al. 1998). In brief, flow-sorted whole-chromosome-painting probes were amplified and labeled in combination, by a stringent DOP-PCR. The M-FISH probe mix was hybridized on metaphase spreads, and images were captured by the Leica DMRXA-RF8 epifluorescence microscope equipped with a Photometrics Sensys CCD camera. The Leica MCK software package (Leica Microsystems Imaging Solutions) was used for image analysis.

## Results

### Clinical Picture

Mental retardation segregated in this five-generation pedigree in an autosomal dominant fashion with incomplete penetrance (fig. 1). An increasing number of affected individuals in the younger generations was observed: all 10 patients appeared in generations IV and V, whereas 13 obligate carriers in generations I-IV were asymptomatic. In addition to mild-to-moderate mental retardation, variable signs of nonspecific developmental abnormalities were present, including down-slanting palpebral fissures, hypertelorism, short toes, broad or prominent nasal bridge, talipes equinovarus, and hypospadias (table 1 and fig. 2). On the basis of this clinical spectrum, a clinical diagnosis could not be made.



**Figure 1** Pedigree of family and haplotype analysis for markers at chromosomes 16p13.3 and 3q29. The gray bars represent chromosome 3q sequences; the white bars, 16p sequences.

**Table 1**

**Clinical Characteristics of Patients**

	IV-6		IV-7		IV-8		IV-13		IV-15		IV-17		V-1		V-3		V-5		V-7		
Sex	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	
Age at examination (years)	54	37	40	33	28	27	12	14	14	18	14	18	14	14	18	14	14	18	14	14	
Mental retardation (IQ)	Moderate/severe	Mild	Moderate	Mild	Mild	Mild	Moderate	Mild (65)	Mild (65)	Mild/moderate	Mild (65)	Mild/moderate	Mild/moderate	Mild/moderate	Mild/moderate	Mild/moderate	Mild/moderate	Mild/moderate	Mild/moderate	Mild/moderate	Mild/moderate
Sitting	ND	ND	ND	18 mo	6-7 mo	Delayed	10 mo	12 mo	12 mo	9-10 mo	12 mo	9-10 mo	10 mo	12 mo	9-10 mo	10 mo	9-10 mo	10 mo	10 mo	10 mo	10 mo
Walking	ND	ND	ND	36 mo	18 mo	Delayed	24 mo	30 mo	30 mo	22 mo	30 mo	22 mo	16 mo	30 mo	22 mo	16 mo	22 mo	22 mo	16 mo	16 mo	16 mo
Reading	Absent	Present	Present	Present	Present	Present	Little	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
Writing	Absent	Little	Present	Little	Present	Present	Absent	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
First words	Delayed	ND	ND	2 years	1.5 years	1.5 years	3 years	2 years	2 years	2.5 years	2 years	2.5 years	3 years	2 years	2.5 years	3 years	2.5 years	2.5 years	2.5 years	2.5 years	2.5 years
Hypotonia, postnatal	ND	ND	ND	Absent	Present	Severe	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
Opisthotonus	ND	ND	ND	Absent	Absent	ND	Absent	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Birth weight/length <sup>a</sup>	ND	ND	ND	3,070 g/50 cm	2,760 g/48 cm	2,350 g	2,780 g/48 cm	3,480 g/49 cm	3,480 g/49 cm	2,910 g/49 cm	3,480 g/49 cm	2,910 g/49 cm	2,900 g/48 cm	3,480 g/49 cm	2,910 g/49 cm	2,900 g/48 cm	2,910 g/49 cm	2,910 g/49 cm	2,900 g/48 cm	2,900 g/48 cm	2,900 g/48 cm
Failure to thrive/tube feeding	ND	ND	ND	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
Down-slanting palpebral fissures	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
Hypertelorism	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
Nasal bridge	Absent	Broad	Broad	Prominent	Broad	Broad	Broad	Broad	Broad	Broad	Broad	Broad	Broad	Broad	Broad	Broad	Broad	Broad	Broad	Broad	Broad
Foot deformation	TE	TE	TE	Absent	TE	TE	Pes adductus	TE	TE	TE	TE	Pes adductus	TE	TE	Pes adductus	TE	Pes adductus	Pes adductus	Pes adductus	Pes adductus	Pes adductus
Short toes	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present	Present
Hypospadias	ND	ND	ND	Present	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Undescended testes	Present	NR	ND	ND	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR

NOTE.—ND = not determined; NR = not relevant; TE = Talipes equinovarus.

<sup>a</sup> All were born at term.



**Figure 2** Facial photograph of patient V-5. Note hypertelorism, broad nasal bridge, and down-slanting palpebral fissures but absence of specific dysmorphic features.

#### *Evaluation for Mental Retardation*

Conventional G-banded karyotyping at the subband level of ~600, high-resolution G-banded karyotyping at the subband level of ~800, and M-FISH analysis (Speicher et al. 1996) showed no evidence of karyotypic abnormalities in either of the two affected patients, IV-13 or IV-15. Results of fragile X testing were negative, and neuroimaging and metabolic testing showed no evidence of any specific abnormality.

#### *Linkage Analysis*

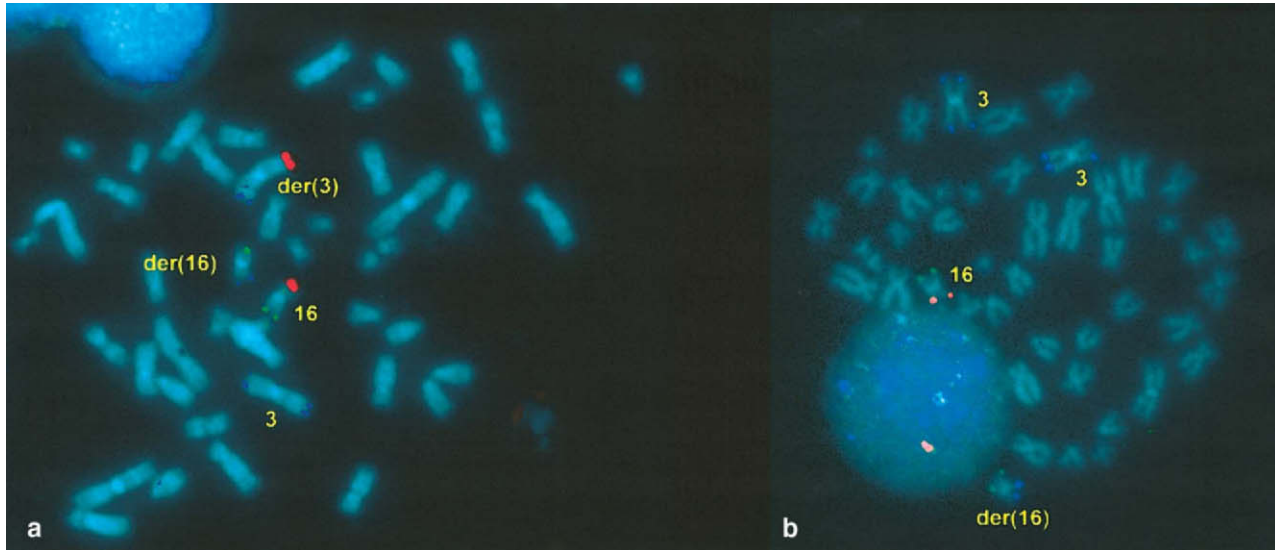
Therefore, DNA from the patients, the obligate carriers, and their spouses was analyzed with the aid of the CHLC fluorescein-labeled human screening set, and significant linkage (LOD score 4.13 at  $\theta = 0$ ) was found with marker D16S2622 at chromosome 16p13.3. Nine additional markers from this region from the Généthon human linkage map, spanning a 16-cM (4.8 Mb, according to the Genetic Location Database) region, were analyzed, and linkage was confirmed (table 1). A proximal recombinant was found with marker D16S3030. Surprisingly, the distal markers D16S3024 and D16S521 in a 5-cM (2.7 Mb) region, and an additional marker—HBA2—between D16S521 and D16S3042, were hemizygous in all patients examined.

In none of the patients was a parental disease allele detected with any of these three markers, indicating a deletion of terminal 16p13.3 sequences, with a breakpoint between markers D16S3024 and D16S3124, in a 0.6-cM (0.1Mb) region (fig. 1).

#### *Detection of Cryptic Translocation by FISH*

FISH was performed on transformed cells of patient IV-13, with PAC clone 27M3, which contained the D16S3024 locus for which the patients were hemizygous. The PAC clone 27M3 hybridized only to one 16p13.3 homologue in 50 metaphases examined; cosmid CosRT1, derived from the Rubenstein-Taybi syndrome locus (which is retained in the patients), hybridized to both 16p13.3 homologues. This indicated that the mental retardation in this family might be explained by a deletion of the tip of chromosome 16p.

To detect whether the 16p deletion was due to a translocation, FISH analysis was performed on metaphase spreads of suspected translocation carriers III-10 and IV-4, with subtelomeric 16p probes 27M3 and 119L16, respectively. In these carriers, both 16pter probes hybridized to the tip of one copy of chromosome 16p, as well as to the tip of chromosome 3q (fig. 3a). This indicated a cryptic balanced subtelomeric microtranslo-



**Figure 3** a, Balanced translocation  $t(3;16)(q29;p13.3)$  in carrier IV-4. Metaphase spread was hybridized with subtelomere probes 3p and 3q (blue), 16q (green), and 16p (red). b, Unbalanced karyotype in individual V3. Metaphase spread was hybridized with subtelomere probes 3p and 3q (blue), 16q (green), and 16p (red) show trisomy of the tip of 3q and monosomy of the tip of 16p.

cation,  $t(3;16)(q29;p13.3)$ , segregating in this family. Subsequent FISH analysis with the subtelomeric 3q probe 196F4 confirmed the presence of 3qter sequences on 16pter in carrier IV-4. In patient V-5, deletion 16pter and duplication 3qter were observed (fig. 3b).

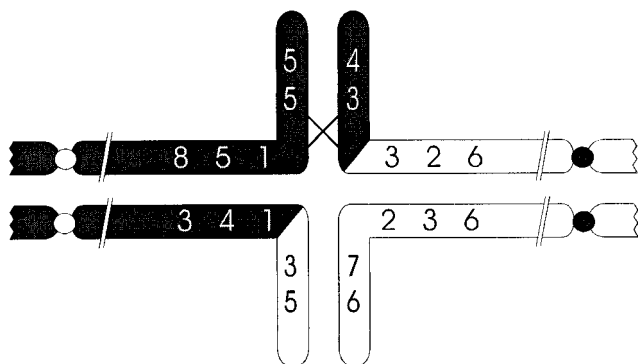
To determine the precise location of the translocation breakpoint on 16p13.3, FISH analysis with cosmids from a contig covering this region was performed (Nellist et al. 1993). This allowed us to narrow the translocation breakpoint on chromosome 16 to between cosmid clone cC1-2 and cCBSF1. Thus, it can be estimated

that the translocated part encompasses  $\leq 2$  Mb of 16p13.3.

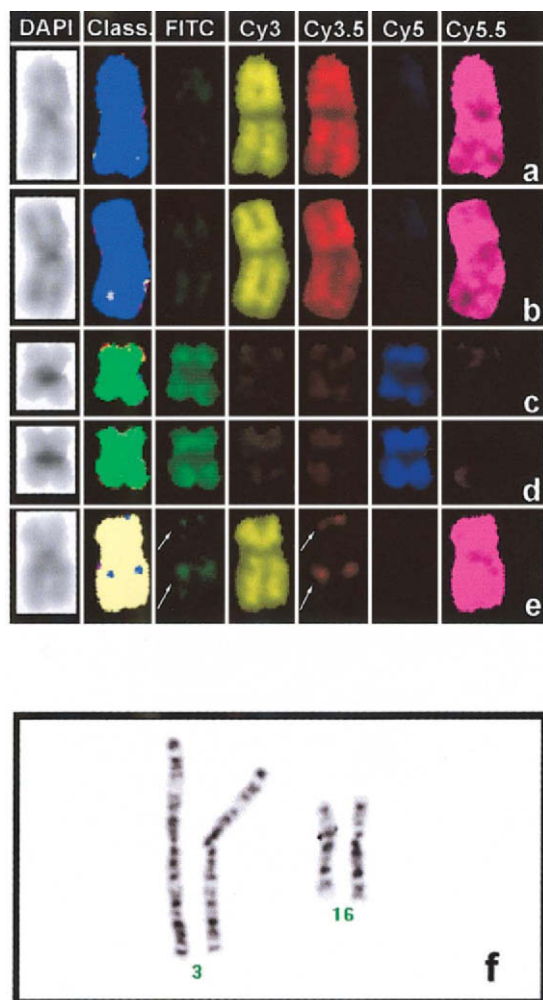
#### Chromosome 3q29 Haplotyping

Subsequently, markers on terminal 3q29 of the Génethon map were analyzed in this family to determine both the segregation of the translocation in this family and the extent of the translocated part of chromosome 3 on the genetic map. Markers D3S1305 and D3S3550 resulted in triallelic haplotypes in all patients examined, whereas markers D3S1601, D3S3669, and D3S2748 showed conventional segregation (fig. 1). In combination with the FISH results described in the last paragraph, these findings confirmed that the cryptic balanced subtelomeric translocation segregated in this whole family, resulting in an unbalanced karyotype with partial deletion 16p13.3 and partial duplication 3q29 in patients. The translocation breakpoints on chromosome 3q29 occurred between markers D3S2748 and D3S1305, a 2.4-cM (0.5Mb) region. The translocated part encompasses a region of  $\leq 3$  Mb.

A recombination event between the translocated part of chromosome 3 on chromosome 16 and the normal chromosome 3 was observed in carrier IV-4. This is schematically represented in figure 4. Crossing-over between a translocation chromosome and a normal chromosome must have occurred during meiosis I of carrier father III-1, when the two translocated chromosomes and their normal counterparts come together as a quadrivalent. In this configuration, the translocated part of chromo-



**Figure 4** Pachytene analysis of the quadrivalent complex of the translocated and the normal chromosomes during meiosis I. The cross-over that is noticed in individual IV-4 is indicated. This recombination explains why the 3q haplotype in patient IV-4 is different from that in the other patients.



**Figure 5** Retrospective partial karyotypes of carrier IV-5, showing no abnormalities on tips of chromosome 3q and 16p. The two chromosomes 3 (*a* and *b*) and 16 (*c* and *d*) and one X chromosome (*e*) from one of the carriers of the balanced t(3;16) after M-FISH hybridization are shown. The first column shows the inverted DAPI image, the second column the chromosomes in classification colors. Columns three to five display the individual fluorochrome channels. Chromosome 3 is labeled with a combination of Cy3, Cy3.5, and Cy5.5, chromosome 16 with a combination of FITC and Cy5. Therefore, on one of the two long arms of chromosome 3, additional bands in the FITC and Cy5 channels should be visible and, correspondingly, one of the short arms of chromosome 16 should display additional bands in the Cy3, Cy3.5, and Cy5.5 channels. However, none of these bands are visible. As an approximate measure for the resolution, an X chromosome from the same metaphase spread is shown (*e*). Because of the different labeling of the X chromosome (Cy3 and Cy5.5) and the Y chromosome (FITC, Cy3.5, and Cy5.5) in the M-FISH mix, additional bands are visible on the X chromosome in the FITC and Cy3.5 channels. These bands represent the first pseudoautosomal region at Xp22.3 (size 2.6 Mb *small arrows*) and the XY homology region at Xq21.3 (size 4 Mb *large arrows*). The second pseudoautosomal region at Xq28 is too small (320 kb) to be detected. High-resolution banding of chromosomes 3 and 16 (*f*) demonstrates that the translocation is cytogenetically invisible.

some 3 on the der(16) was exchanged with the corresponding part of the intact chromosome 3 copy.

#### *Subnormal Hematologic Values in Patients*

Since the HBA2 marker at the  $\alpha$ -globin-cluster region is deleted in patients, retrospective hematologic studies of blood samples of patients V-5 and V-7 were performed. This study showed subnormal red-blood-cell parameters for mean corpuscular hemoglobin (MCH) and hemoglobin concentration (MCH 22.4 and 21.2 pg, and Hb 12.6 and 11.5 g/dl, for V-5 and V-7, respectively), whereas the values of their parents, IV-9 and IV-10 (MCH: 30.1 and 31.4 pg, Hb 13.8 and 13.0 g/dl, for IV-9 and IV-10, respectively), were within the normal range (normal values for male and female adults are Hb 13.5–17.5 g/dl and 12–16 g/dl, respectively; mean cell Hb (MCH) 26–34 pg). These hematologic values are compatible with the thalassemia trait, a subclinical form of  $\alpha$ -thalassemia caused by loss of one copy of the  $\alpha$ -globin cluster.

#### *Retrospective High-Resolution Karyotyping and M-FISH*

To verify whether the translocation could be detected in retrospect by cytogenetic analysis, we made a new high-resolution karyotype and performed M-FISH analysis of the carrier of the balanced t(3;16) translocation (IV-5). However, even though special attention was given to the tips of the short arm of chromosome 16 and to the long arm of chromosome 3, in none of them was the translocation detectable (fig. 5).

#### **Discussion**

We have shown that a balanced cryptic subtelomeric translocation segregates in this family for at least five generations. It led to 10 cases with both terminal duplication of 3q29 and terminal deletion of 16p13.3. This unbalanced karyotype is a product of an adjacent-1 segregation of a cryptic reciprocal subtelomeric translocation, t(3;16)(q29;p13.3). The translocated sequence encompasses  $\leq 2$  Mb of the top of chromosome 16p and  $\leq 3$  Mb of the top of chromosome 3q.

Nine cases of subtelomeric deletions of the short arm of chromosome 16 that were similar in size to the one detected in the family reported here have been described. Five patients had a seemingly de novo deletion (Wilkie et al. 1990a, 1990b; Lindor et al. 1997), whereas the remaining four patients had an unbalanced translocation with both terminal deletion of 16p13.3 and terminal duplication of different translocation partner chromosomes—namely, 1p36.3, 15q13.1, and 10q26.13 (Wilkie et al. 1990a). A combination with terminal duplication

3q29, as observed in the family studied, has not been described.

Deletion of terminal 16p13.3, also called “ATR-16 syndrome,” is defined as a contiguous-gene syndrome resulting from chromosomal rearrangements that delete the  $\alpha$ -globin genes and a putative mental retardation gene, among a number of other genes (Weatherall et al. 1981; Wilkie et al. 1990a). Because of the restricted number of patients, the phenotypic range of ATR-16 syndrome still is ill defined, but the most frequently observed features besides mental retardation and some form of  $\alpha$ -thalassemia include hypertelorism, talipes equinovarus (Wilkie et al. 1990a), down-slanting palpebral fissures, a broad flat nasal bridge, and epicanthic folds (Lindor et al. 1997). Since the clinical features of the affected individuals in the family reported here resemble those of the previously described cases of ATR-16 syndrome, we consider these 10 cases of a terminal deletion 16p13.3 and terminal duplication 3q29 as 10 new cases of ATR-16 syndrome. The most consistent features in the family members studied were relatively low birth weight, hypotonia, pes equinovarus in both sexes, and undescended testes in the males. The facial dysmorphism is mild.

Duplication of such a small part of the long arm of chromosome 3, as in the family studied, has never been described. The 3q duplication associated with the Cornelia DeLange syndrome (MIM 122370) is proximal to and does not overlap with the trisomic part of chromosome 3, which was seen in our patients. Duplication of a large part of 3q involving chromosome bands 3q21-qter and 3q25-qter has been occasionally observed (Kondo et al. 1979; Garcia-Esquivel et al. 1987; Montero et al. 1988). Clinical features of this patient include mental or developmental retardation and growth failure, hypotonia, persistent lanugo, distorted head, short and upturned nose, prominent maxilla, micrognathia, short limbs, retroflexed third and fourth toes, and coetaneous syndactyly of the second, third, and fourth toes (Kondo et al. 1979). These symptoms do not seem compatible with the patients in the family studied, and the influence of this much smaller terminal duplication 3q on their phenotypic spectrum is hard to define.

Adjacent-1 segregation of this cryptic translocation predicts two different unbalanced karyotypes. However, the reciprocal unbalanced karyotype with terminal deletion 3q and terminal duplication 16p was not detected in our family. Although there are several reports of live-born patients with partial 16p duplication (Léonard et al. 1992; O'Connor and Higgins 1992) and four reports of live-born patients with partial 3q deletion (Alvarez Arratia et al. 1984; Sargent et al. 1985; Brueton et al. 1989; Jokiahio et al. 1989), we propose that an unbalanced karyotype with a combination of both chromosome abnormalities is somehow selected against. Since

there is no history of an increased rate of spontaneous abortion in this family, there might be prezygotic selection against gametes carrying such unbalanced chromosomal complements. FISH analysis on semen of translocation carriers could determine whether such a selection mechanism exists.

The different ratio, between the older and the younger generations, in balanced versus unbalanced segregation of the translocation is remarkable. In generations I–III, there are significantly more balanced segregations, because of an alternate segregation in meiosis I (13 meioses give rise only to balanced karyotypes). In these three generations, no unbalanced translocations are observed. In generations IV and V, there is a 1:1 ratio between balanced and unbalanced segregation (18 meioses give rise to 8 balanced karyotypes and 10 unbalanced karyotypes). A sex difference to explain this discrepancy between the first three and the last two generations could not be noticed. The increased number of unbalanced segregations in the younger generations explains the seeming anticipation initially noticed in this family.

Cytogenetically invisible unbalanced translocations have also been reported to cause other mental retardation syndromes, including Wolf-Hirschhorn syndrome (MIM 194190) (deletion of terminal 4p; Altherr et al. 1997), cri-du-chat syndrome (MIM 123450) (deletion of terminal 5p; Overhauser et al. 1989), and Miller-Dieker syndrome (MIM 247200) (deletion of terminal 17p; Kuwano et al. 1991). In all these syndromes, other phenotypic characteristics, in addition to the mental retardation, contributed to the recognition of the disorder and to the identification of the deletions. Distinguishing phenotypic characteristics were not obvious in the patients from the family studied. Although ATR-16 patients have relatively specific, albeit probably underrecognized, associated findings—for example,  $\alpha$ -thalassemia—the  $\alpha$ -thalassemia trait was recognized only retrospectively and played no role in the identification of the ATR-16 syndrome in this family. Moreover, M-FISH, which, on the basis of GTG-banding, is able to detect cryptic translocations in supposedly normal metaphase spreads (Uhrig et al. 1999), in this case failed to identify the translocation. As documented in figure 5, the translocation did not result in additional bands at the respective derivative chromosomes, and, therefore, the classification algorithm could not detect this exchange of chromosomal material. In general, the X chromosome is used as an approximate measure for the resolution and the hybridization quality. As stated elsewhere (Uhrig et al. 1999), the consistent detection of the first pseudoautosomal region at Xp22.3 and of the concurrent absence of the second at Xq28 (fig. 5e) sets the resolution limits to 320 kb–2.6 Mb. The fact that the first pseudoautosomal region was clearly identified, even though the translocated material did not yield extra signals on



the derivative chromosomes, could be because the size of the exchanged chromosomal material in this family is below the detection limit of 2.6 Mb but might also be because of the fact that the telomeric regions are underrepresented in the repeat-depleted probes used for M-FISH hybridization.

It is, therefore, crucial to realize that, if this family were not of a size large enough for linkage analysis, these patients would simply have been designated as having idiopathic mental retardation. The decision of whether to evaluate cryptic subtelomeric chromosomal rearrangement in patients with mental handicap is very difficult to make. Although it is estimated that 5%–10% of all cases of idiopathic mental retardation have a small subtelomeric rearrangement (Flint et al. 1995; Girau-deau et al. 1997; Slavotinek et al. 1999), the number of patients identified with this etiology remains small.

Therefore, this case of ATR-16 syndrome, in which the segregating translocation was initially not noticed with the aid of standard cytogenetic analysis and M-FISH, once more points out the importance of subtelomeric chromosomal microrearrangements in idiopathic mental retardation, not only in sporadic cases or in small families but also in large families, irrespective of the suspected inheritance pattern. Thus, a need is indicated for reliable and fast screening sets for the subtelomeric regions, either by FISH or by microsatellite markers.

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## Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

The Genetic Location Database, [http://cedar.genetics.soton.ac.uk/public\\_html](http://cedar.genetics.soton.ac.uk/public_html) (for inferred physical location of loci markers)

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim> (for Cornelia DeLange syndrome [MIM 122370], Wolf-Hirschhorn syndrome [MIM 194190], cri-du-chat syndrome [MIM 123450], and Miller-Dieker syndrome [MIM 247200])

## References

- Altherr MR, Wright TJ, Denison K, Perez-Castro AV, Johnson V (1997) Delimiting the Wolf-Hirschhorn syndrome critical region to 750 kilobase pairs. *Am J Med Genet* 71:47–53
- Alvarez Arratia MC, Rivera H, Möller M, Valdivia A, Viguera A, Cantu JM (1984) De novo del(3)(q28). *Ann Genet* 27:109–111
- Brueton LA, Barber JCK, Huson SM, Winter RM (1989) Partial monosomy 3q in a boy with short stature, developmental delay, and mild dysmorphic features. *J Med Genet* 26:729–730
- Curry CJ, Stevenson RE, Aughton D, Byrne J, Carey JC, Cassidy S, Cunniff C, et al (1997) Evaluation of mental retardation: recommendations of a consensus conference. *Am J Med Genet* 72:468–477
- Dib C, Fauré S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, et al (1996) A comprehensive genetic map of the human genome based on 5,264 microsatellites. *Nature* 380:152–154
- Eils R, Uhrig S, Saracoglu K, Sätzler K, Bolzer A, Petersen I, Chassery J, et al (1998) An optimized, fully automated system for fast and accurate identification of chromosomal rearrangements by multiplex-FISH (M-FISH). *Cytogenet Cell Genet* 82:160–171
- Flint J, Wilkie AOM, Buckle VJ, Winter RB, Holland AJ, McDermid HE (1995) The detection of subtelomeric chromosomal rearrangements in idiopathic mental retardation. *Nat Genet* 9:132–139
- Garcia-Esquivel L, Rivas F, Rivera H, Möller M, Velazquez AL, Cantu JM (1987) Familial trisomy 3q25-qter: report of two cases. *J Genet Hum* 35:299–303
- Giraudeau F, Aubert D, Young I, Horsley S, Knight S, Kearney L, Vergnaud G, et al (1997) Molecular-cytogenetic detection of a deletion of 1p36.3. *J Med Genet* 34:314–317
- Jokiaho I, Salo A, Niemi KM, Blomstedt GC, Pihkala J (1989) Deletion 3q27-3qter in an infant with mild dysmorphism, parietal meningocele, and neonatal miliaria rubra-like lesions. *Hum Genet* 83:302–304
- Kondo I, Hirano T, Hamaguchi H, Ohta Y, Haibara S, Nakai H, Takita H (1979) A case of trisomy 3q21 leads to qter syndrome. *Hum Genet* 46:141–147
- Kuwano A, Ledbetter SA, Dobyns WB, Emanuel BS, Ledbetter DH (1991) Detection of deletions and cryptic translocations in Miller-Dieker syndrome by in situ hybridization. *Am J Hum Genet* 49:707–714
- Lathrop GM, Lalouel JM (1984) Easy calculations of lod scores and genetic risks on small computers. *Am J Hum Genet* 36:460–465
- Léonard C, Huret JL, Imbert M-C, Lebouc Y, Selva J, Bouley A-M (1992) Trisomy 16p in a liveborn offspring due to maternal translocation t(16;21)(q11;p11) and review of the literature. *Am J Med Genet* 43:621–625
- Lindor NM, Valdes MG, Wick M, Thibodeau SN, Jalal S (1997) De novo 16p deletion: ATR-16 syndrome. *Am J Med Genet* 72:451–454
- Montero MR, Martinez A, Fayos JL, Alvarez V (1988) A new case of partial trisomy 3(q25-qter) in a newborn. *Ann Genet* 31:65–68
- Nellist M, Janssen B, Brook-Carter PT, Hesselting-Janssen

- ALW, Maheshwar MM, Verhoef S, van den Ouweland AMW, et al (1993) Identification and characterization of the tuberous sclerosis gene on chromosome 16. *Cell* 75: 1305–1315
- Ning Y, Roschke A, Smith ACM, Macha M, Precht K, Riethman H, Ledbetter DH, et al (1996) A complete set of human telomeric probes and their clinical application. *Nat Genet* 14:86–89
- O'Connor TA, Higgins RR (1992) Trisomy 16p in a liveborn infant and review of trisomy 16p. *Am J Med Genet* 42: 316–319
- Overhauser J, Bengtsson U, McMahon J, Ulm J, Butler MG, Santiago L, Wasmuth JJ (1989) Prenatal diagnosis and carrier detection of a cryptic translocation by using DNA markers from the short arm of chromosome 5. *Am J Hum Genet* 45:296–303
- Sargent C, Burn J, Baraitser M, Pembrey ME (1985) Trigonoccephaly and the Opitz C syndrome. *J Med Genet* 22:39–45
- Slavotinek A, Rosenberg M, Knight S, Gaunt L, Fergusson W, Killoran C, Clayton-Smith J, et al (1999) Screening for submicroscopic chromosome rearrangements in children with idiopathic mental retardation using microsatellite markers for the chromosome telomeres. *J Med Genet* 36:405–411
- Speicher MR, Ballard SG, Ward DC (1996) Karyotyping human chromosomes by combinatorial multi-fluor FISH. *Nat Genet* 12:368–375
- Uhrig S, Schuffenhauer S, Fauth C, Wirtz A, Daumer-Haas C, Apacik C, Cohen M, et al (1999) Multiplex-FISH for pre- and postnatal diagnostic applications. *Am J Hum Genet* 65: 448–462
- Weatherall DJ, Higgs DR, Bunch C, Old JM, Hunt DM, Presley L, Clegg JB, et al (1981) Hemoglobin H disease and mental retardation: a new syndrome or a remarkable coincidence? *N Engl J Med* 305:607–612
- Wilkie AOM, Buckle VJ, Harris PC, Lamb J, Barton NJ, Reeders ST, Lindenbaum RH, et al (1990a) Clinical features and molecular analysis of the  $\alpha$  thalassemia/mental retardation syndromes. I: Cases due to deletions involving chromosome band 16p13.3. *Am J Hum Genet* 46:1112–1126
- Wilkie AOM, Lamb J, Harris PC, Finney RD, Higgs DR (1990b) A truncated human chromosome 16 associated with  $\alpha$  thalassemia is stabilized by addition of telomeric repeat (TTAGGG)<sub>n</sub>. *Nature* 346:868–871