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# Identification of Cryptic Rearrangements in Patients with 18q− Deletion Syndrome

Zoran Brkanac,[1] Jannine D. Cody,[1] Robin J. Leach,[1,2] and Barbara R. DuPont[1]

Departments of [1]Cellular and Structural Biology and [2]Pediatrics, University of Texas Health Science Center at San Antonio, San Antonio

## Summary

**The majority of patients with 18q− syndrome appear cytogenetically to have a terminal deletion of the long arm of chromosome 18. These 18q− patients are diagnosed by use of standard cytogenetic banding techniques, which have resolution insufficient for precise genotyping. In our effort to obtain a thorough genotype, we have analyzed the DNA from 35 patients who originally were diagnosed as having de novo terminal deletions of chromosome 18. Molecular analysis was performed with polymorphic markers throughout the 18q− region. Cytogenetic FISH was performed with two human 18q telomeric probes, a chromosome 18–specific α-satellite probe, and whole chromosome 18–specific paint. Of 35 patients previously reported to have terminal deletions of 18q, we found that 5 (14%) have more-complex cryptic rearrangements and that 3 (9%) retain the most distal portion of 18q, consistent with an interstitial rather than a terminal deletion. These findings indicate that a standard karyotype can lead to insufficient characterization in 18q− syndrome. This has important ramifications for phenotype mapping of this syndrome, as well as for proper prognosis.**

## Introduction

The 18q− syndrome is a partial aneusomy disorder resulting from the deletion of a portion of the long arm of chromosome 18. Since 1964, when it was first described by de Grouchy et al. (1964), it has been found to be one of the more common autosomal-chromosomal deletion syndromes (Schinzel 1984). Patients with 18q− syndrome exhibit a wide range of features. Among the more common features are developmental delay, mental retardation, incomplete myelination, microcephaly, facial and limb abnormalities, genitourinary malformations, neurological abnormalities, hearing abnormalities, and growth failure (Schinzel 1984; Miller et al. 1990). A more comprehensive list of clinical manifestations reveals a broad phenotypic spectrum with many other features found less frequently (Strathdee et al. 1995). A wide variability in clinical presentation of 18q− syndrome is not surprising, since all known aneuploidies are highly variable (Brown et al. 1993; Penny et al. 1995). This may be due to genetic background, imprinting, extent (size and localization) of deletions, or other chromosomal alterations. For other aneusomies, it has been shown that chromosomal regions can be identified and correlated with specific clinical presentations (Korenberg et al. 1990; Penny et al. 1995). Such an effort requires precise genotypic analysis coupled with extensive clinical investigation.

Initial efforts to evaluate patients with 18q deletions at the clinical, cytogenetic, and molecular levels, for the purpose of phenotypic mapping, have already been reported. Kline et al. (1993), using a molecular and cytogenetic approach, established a correlation between the extent of deletion and the clinical picture in seven patients. These correlations were not confirmed in the evaluation, by the same group, of an additional 19 patients (Strathdee et al. 1995). These investigators also have performed molecular analyses of the ends of the deleted chromosomes (Strathdee et al. 1994, 1995) and have found only terminal deletions in their patient population. Other investigators have identified, by cytogenetic means, individuals with interstitial deletions of 18q (Wilson et al. 1979; Chudley et al. 1992; Krasikov et al. 1992). It is important to determine whether deletions are terminal or interstitial, because the ends of the chromosomes have been found to be rich in transcribed sequences (Saccone et al. 1992). Physically small but transcriptionally rich undetected terminal sequences can greatly complicate phenotypic mapping.

In this report, we describe the results of detailed analysis performed on 35 patients previously reported, by routine cytogenetic methods, to have terminal deletions of 18q. To obtain a more precise genotype, we have used

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Address for correspondence and reprints: Dr. Barbara R. DuPont, Department of Cellular and Structural Biology, University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78284. E-mail: dupont@uthscsa.edu

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molecular analysis, high-resolution G-banding, and FISH to analyze our patients. We have found five individuals with more-complex cryptic rearrangements. Detection of patients with complex structural abnormalities will result in both a more accurate prognosis and a better understanding of the genetic heterogeneity of this syndrome.

## Patients and Methods

### *Patient Population*

Patients were referred either by their individual physicians or from the Chromosome 18 Registry and Research Society, a support group for families of individuals with chromosome 18 abnormalities. The study was approved by the institutional review board at the University of Texas Health Science Center at San Antonio, and informed consent was obtained from all subjects.

### *PCR Amplification*

Molecular analysis to confirm the deletions of material from the long arm of chromosome 18 was performed by use of PCR-based microsatellite markers (Dib et al. 1996). To obtain DNA for this analysis, blood samples were obtained from the patient and both parents (when available). High-molecular-weight genomic DNA was extracted from the peripheral blood leukocytes by the method of Bell et al. (1981). The DNA from each family was analyzed by use of as many as 18 PCR-based markers. These markers were originally identified by Généthon and have been placed in a linear order (Dib et al. 1996). PCR was performed in a total reaction volume of 10 $\mu$l, with 50 ng of genomic DNA, 50 ng of each primer, 200 mM of each dNTP, and 0.5 U of *Taq* polymerase (Perkin Elmer Cetus). Magnesium levels and annealing temperatures were optimized for each set of primers. One primer of each pair was end labeled at the 5′ end, with $\gamma[^{32}P]$- dATP. PCR amplification consisted of 30 cycles of 1 min at 95°C, followed by 1 min at the appropriate annealing temperature and 1 min elongation at 72°C. PCR products were separated on a 7% polyacrylamide gel run at 65 W for 4–6 h and were visualized by means of Kodak XAR-5 film with intensifying screens.

### *Pulsed-Field Gel Electrophoresis*

Agarose plugs containing DNA from the human telomeric YAC clone yRM2050 were prepared as described elsewhere (Riethman et al. 1989; Dracopoli et al. 1994). The samples were electrophoresed in 0.5 × Tris-borate EDTA buffer chilled to 14°C, with a voltage gradient of 6 V/cm and a switch time of 30–45 s, for 23 h, with a CHEF-DR III Pulsed Field Electrophoresis System (Bio-Rad Laboratories). Gels were stained in ethidium bromide and were visualized, and the band containing the human insert DNA was excised. The YAC containing the human DNA was purified from the gel by use of the Prep-A-Gene DNA Purification System (Bio-Rad Laboratories) and was quantified with a DNA Dip Stick Kit (Invitrogen).

### *P1 Genomic-Clone Isolation*

A human genomic P1 library constructed in Dr. Sternberg's laboratory was obtained from DuPont (Shepherd et al. 1994). This library is arrayed by the strategy first described, by Green and Olson (1990), for YACs. It includes human inserts with a size range of 70–95 kb and contains three to four genome equivalents. PCR primers specific for the marker D18S553 were used to screen the library for clones containing this sequence. This marker is one of the most telomeric markers on chromosome 18q, mapping within the distal 270 kb of 18q, and is contained within the human 18q half-YAC telomere probe, yRM2050. A positive clone was isolated and expanded, and DNA was isolated by use of a modified Qiagen large-scale preparation (Qiagen; modified protocol obtained from Genome Systems).

### *FISH*

Metaphase chromosome spreads were obtained either from Epstein-Barr virus–transformed lymphocytes (Anderson and Gusella 1984) or by primary blood harvest. For blood harvest, preparations were made by standard methods (Moorehead et al. 1960; Ikeuchi 1984), and a similar protocol was used for lymphoblastoid lines (Gibas and Jackson 1985). The YAC DNA and the P1 DNA were labeled, by nick translation, with either biotin-14-dATP (BRL/Gibco) or digoxigenin-11-dUTP (Boehringer Mannheim Biochemical). Slides for FISH, prepared the day before use, were baked for 4 h and were denatured for 2 min (70% formamide and 2 × SSC, pH 7.0). Hybridization buffer (50% formamide, 10% dextran sulfate, and 2 × SSC, pH 7.0), human Cot-1 DNA, chromosome 18 $\alpha$-satellite DNA (Oncor), and 40–100 ng of telomere-region (YAC or P1) probe were mixed, denatured for 5 min, and immediately added to slides. These were allowed to hybridize by overnight incubation at 37°C in a humid chamber. Slides were washed and fluorescently labeled the following day, by means of avidin-conjugated fluorescein isothiocyanate (FITC). For two-color FISH, avidin-conjugated Texas Red and digoxigenin-specific antibodies labeled with FITC were used for labeling the probes. Amplification of the signal was performed with biotinylated anti-avidin and avidin–Texas Red, for the biotin-labeled probe, and with FITC-conjugated anti-sheep antibodies, for the digoxigenin probe. The chromosomes were counterstained with 4′,6-diamidino-2-phenylindole dihydrochloride

**Table 1**

**Summary of Results from Five Patients with Cryptic Rearrangements**

| | Patient 13 | Patient 17 | Patient 30 | Patient 31 | Patient 34 |
|---|---|---|---|---|---|
| Molecular analysis | Interstitial deletion between D18S65 and D18S461 | Terminal deletion distal to D18S450 | No confirmed deletion | Interstitial deletion between D18S447 and D18S70 | Terminal deletion distal to D18S69 |
| High-resolution G-banding | Terminal deletion at q21.1 46,XX, del(18)(q21.1) | Abnormal karyotype 46,XY,der(18) add(18)(q21.3) | Terminal deletion at q21.1 46,XX, del(18)(q21.1) | Terminal deletion at q23 46,XX, del(18)(q23) | Abnormal karyotype 46,XY,der(18) add(18)(q21.3) |
| Two-color FISH with chromosome 18 centromere (green) and 18q telomere (red) | Two green centromere signals, two red terminal signals on each 18q, interstitial deletion on the normal 18q | Two green centromere signals and an interstitial green signal on abnormal 18q, one red interstitial deletion, paracentric inversion | Two green centromere signals, two red terminal signals on each 18q, interstitial deletion | Two green centromere signals, two red terminal signals on each 18q | Two green centromere signals, one red terminal signal on normal 18q, terminal deletion |
| Whole chromosome 18 paint | Entire chromosome painted and no 18 signal elsewhere | Entire chromosome painted with only one centromere and no 18 signal elsewhere | Entire chromosome painted and no 18 signal elsewhere | Entire chromosome painted and no 18 signal elsewhere | Entire chromosome painted and no 18 signal elsewhere |

(DAPI) and were viewed by means of a Zeiss Axioscop fluorescent microscope equipped with FITC, DAPI, Texas Red, and triple-bandpass filter sets. Images were captured by a computer using Applied Imaging Probevision software, and photographs were printed on a Kodak XL 7700 color-image printer. The chromosome 18 paint probe (Cambio) was applied according to the manufacturer's protocol, except that only 2 $\mu$l of the probe was used. For each patient, >20 cells were screened either for the presence of 18q telomeric sequences or for paint distribution.

### *Cytogenetics*

Chromosomes were prepared, in the manner described above, for prometaphase spreads. The chromosome-banding level was determined by the method of Josifek et al. (1991). For each patient, $\geqslant$20 metaphase spreads were counted, and 10 of them, with a band level $\geqslant$550, were analyzed on the microscope. A minimum of five spreads were photographed, and two were karyotyped.

## Results

High-resolution G-banding was performed on all 18q− patients in our study who either had been referred to us without a cytogenetic report, had a previous cytogenetic report that was of insufficient quality, and/or had undergone molecular analysis that showed large uninformative regions. From this analysis, two patients (patients 17 and 34) were found to have cryptic rearrangements. The results of the G-banding studies are described below and are summarized in table 1.

To estimate a proximal breakpoint location, genomic DNA from the 35 families that we studied was analyzed by use of $\geqslant$18 polymorphic PCR-based markers distributed throughout the 18q− region. Multiple markers along the long arm were used to confirm absence of a copy of chromosome 18 material. All 35 patient samples were analyzed with the most distally placed marker, D18S70 (also known as "D18S497"), which maps within the distal 270 kb of 18q and is contained within the human 18q half-YAC clone yRM2050. The DNA samples from 24 patients were informative, and 3 (patients 13, 30, and 31) retained two copies of the marker. The results of the molecular analysis of these three patients are summarized in figure 1 and table 1, along with those for patients 17 and 34. The 11 patients uninformative by molecular analysis with the telomeric PCR marker, a group that included patient 17, were further analyzed by FISH with the half-YAC telomere probe, yRM2050. This analysis revealed no additional patients with interstitial deletions (data not shown).

The five patients identified with apparently more-complicated rearrangements (patients 13, 17, 30, 31, and 34) were studied in greater detail, in order to determine a more precise genotype. Both FISH analysis using two-color FISH with centromere and telomere probes and FISH analysis with whole chromosome 18 paint probe were used to confirm and clarify the previous molecular and cytogenetic findings. The results of these studies for each of our five novel patients are shown in figure 2 and are summarized in table 1. In each section of the figure, the "abnormal" chromosome is on the left, and the "normal" chromosome is on the right. Figure 2*A* shows the results of the high-resolution G-banding. Figure 2*B* shows the results of two-color FISH with chromosome

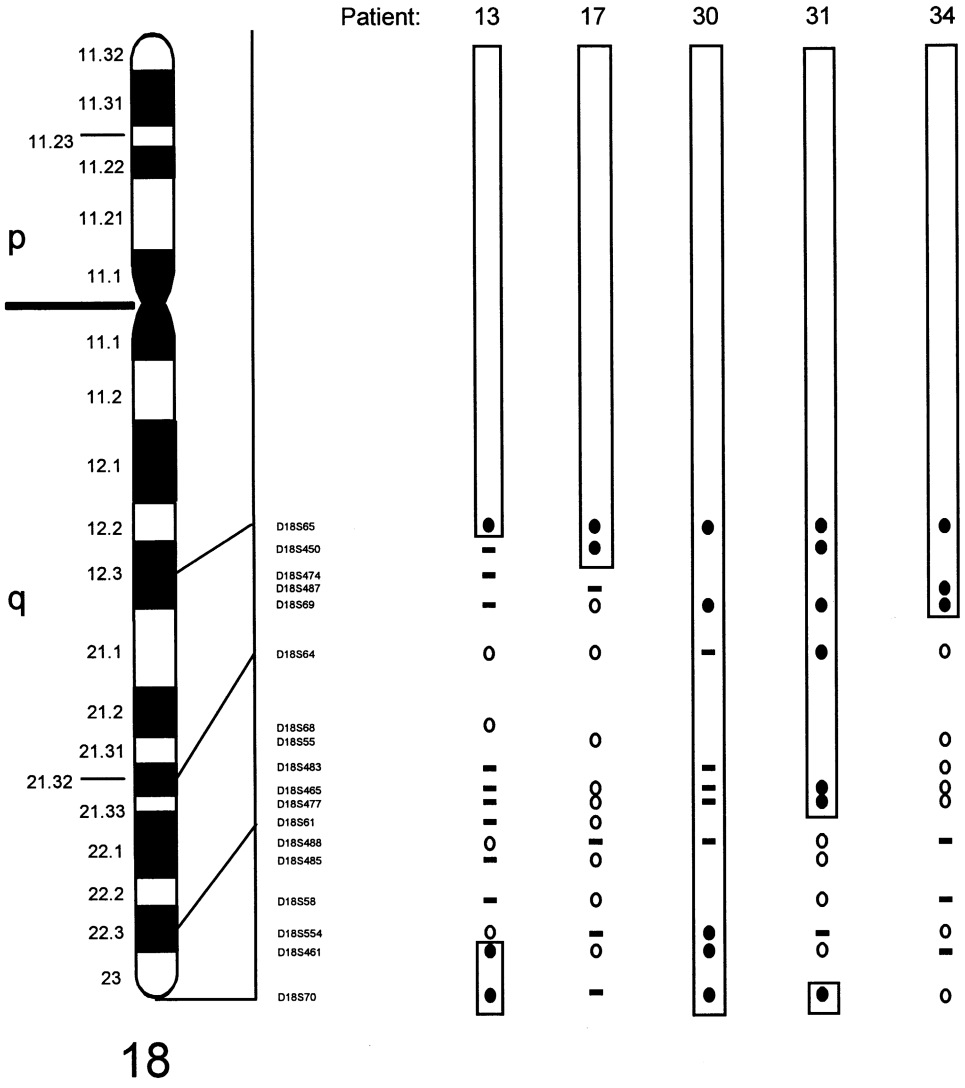


**Figure 1** Molecular analysis of five patients with chromosome 18 deletions, by use of polymorphic markers. A blackened oval indicates that there are two alleles for the corresponding marker; an unblackened oval indicates that the subject has a single allele; and a dash indicates that the marker was uninformative.

18–specific $\alpha$-satellite probe and the human genomic P1 telomere probe, D18S553. This analysis was done to confirm the presence or absence of telomere sequences in these patients. Figure 2*C* shows the chromosome 18s from our five patients examined with whole chromosome 18–specific paint probe (WCP-18). This analysis verifies that these more complex rearranged chromosomes contain only chromosome 18 material and that no chromosome 18 material was translocated elsewhere in the metaphase (data not shown).

Two patients (patients 13 and 31) were found, by molecular analysis, to have interstitial deletions but appeared, by high-resolution G-banding, to have terminal deletions—46,XX,del(18)(q21.1) and 46,XY,del(18)(q23), respectively—of the long arm of chromosome 18. For patient 30, the cytogenetic analysis proved especially useful, since molecular analysis was uninformative for a large region of chromosome 18 and could not confirm any loss of material from this region. The deleted chromosome was found to have a large terminal deletion—46,XX,del(18)(q21.1)—at band q21.1. The analysis of the "normal" chromosome 18 has been more difficult. This chromosome demonstrated an apparently normal banding pattern with a lighter intensity and more diffuse pattern of the terminal bands.

When the chromosomes of these patients were analyzed by two-color FISH, each patient displayed two telomere fluorescent signals and two centromeric signals,

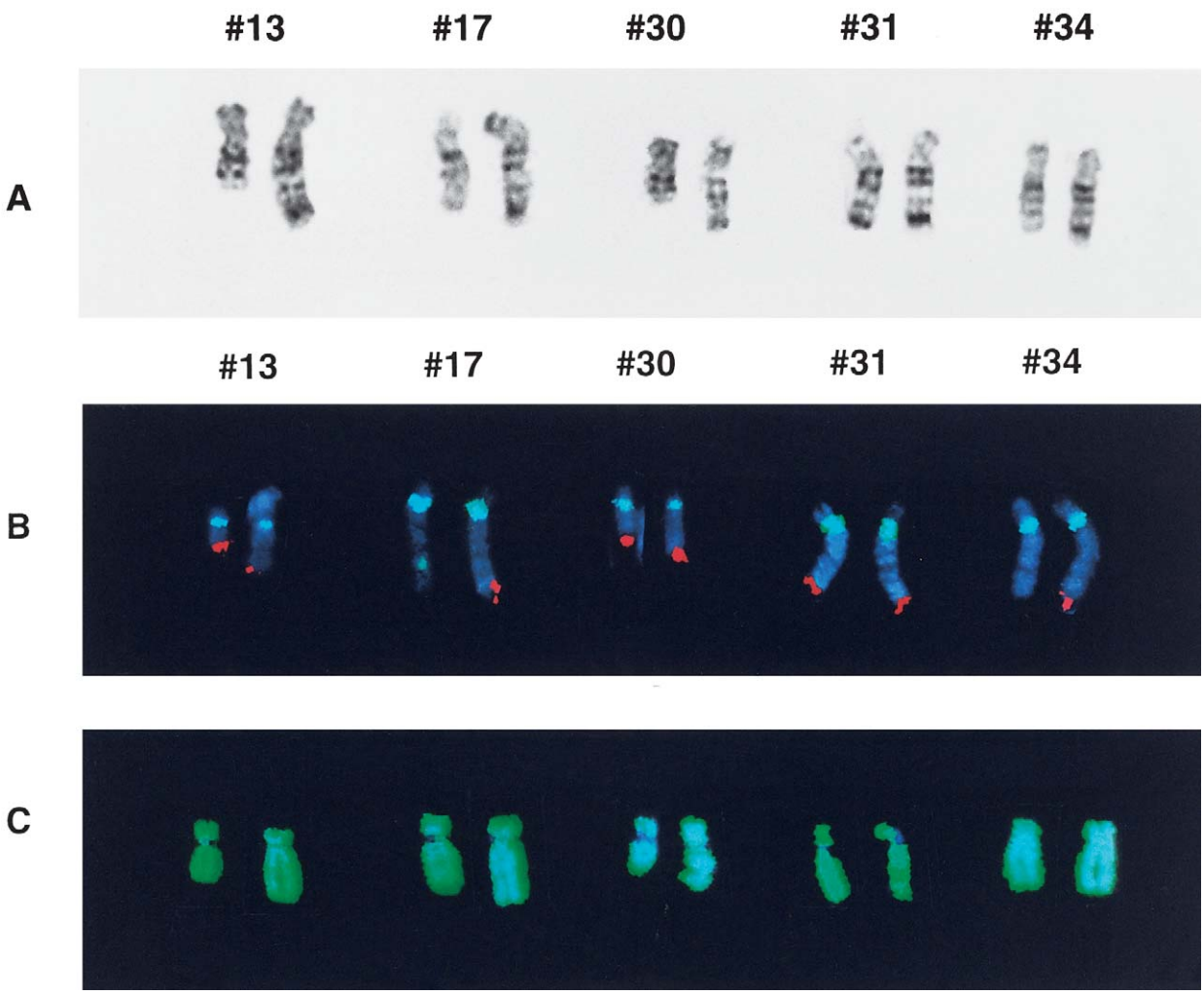


**Figure 2** Cytogenetic and FISH analysis of five patients (identified by the numbers above the chromosome pairs) with chromosome 18 deletions. *A,* High-resolution G-banding. *B,* FISH using FITC-labeled human chromosome 18–specific α-satellite probe (*green*) and Texas Red–labeled human P1 genomic clone (*red*), containing the chromosome 18 telomeric marker D18S553 as the other probe. *C,* FISH with chromosome 18–specific paint labeled with FITC (*green*). All FISH-labeled chromosomes are counterstained with DAPI (*blue*).

confirming the molecular data. Therefore, these patients proved to have interstitial, rather than terminal, deletions. FISH analysis of these five patients with WCP-18 showed only chromosome 18–specific material present on each of the chromosomes, with no chromosome 18 material present elsewhere in the metaphase (data not shown). The "normal" chromosome 18 from patient 30 had an odd "two blob" appearance when whole chromosome 18–specific paint was used, an appearance very much unlike the staining for other normal chromosomes. Further analysis will be needed to determine whether there is an abnormality of this chromosome.

Chromosomes from patients 17 and 34 were found, on the basis of molecular data, to be simple terminal deletions. High-resolution G-banding determined that both were derivative chromosomes from translocations of chromosome 18 with another unknown chromosome—46,XY,der(18)add(18)(q21.3) and 46,XX,der (18)add(18)(q21.3), respectively. The abnormal chromosome from both patients appeared to have large deletions of chromosome 18 material and a wide, euchromatic band at the distal end of the q arm. The two-color FISH analysis of each patient revealed only one copy of the telomere probe, and this was found on the normal chromosome. For patient 17, no telomeric sequences were present on the deleted chromosome, since the molecular analysis was uninformative for this region. One interesting finding was the interstitial localization of a small amount of chromosome 18–specific α-satellite sequence on the long arm of the deleted chromosome of

patient 17. When chromosomes of these two patients were studied with WCP-18, only chromosome 18 material was present on the chromosomes 18, and no chromosome 18 material was seen elsewhere in the metaphase (data not shown). For patient 17, only one centromere was observed in the abnormal chromosome, as demonstrated by only one region of blocked highly repetitive sequence. This indicates that the extra $\alpha$-satellite material in the long arm is not associated with a duplication of the centromere. These results, as well as the size of each derivative chromosome, indicate that each chromosome must also have a partial duplication of chromosome 18 material.

## Discussion

We have described molecular and cytogenetic characterization of patients with 18q− syndrome, which is the sine qua non for phenotypic mapping. Our initial population of 35 patients was referred to us because, by routine cytogenetic methods, they had terminal deletions of 18q. We have reassessed each of these patients by complementary molecular and cytogenetic techniques, to accomplish precise genotypic analysis. Five (14%) of these 35 patients proved to have more-complex cryptic rearrangements.

Three (9%) of the 35 patients retained chromosome 18 telomeric material, as shown by molecular and FISH analysis. Telomeres are known to be rich in transcriptionally active material (Saccone et al. 1992); therefore, small amounts of telomeric genomic material could contain numerous genes and thus greatly influence phenotypic variability. In addition, the shorter chromosome 18 of patient 17 was found, by FISH studies, to have $\alpha$-satellite sequences positioned interstitially and not in association with a duplicated centromere. This suggests an inversion or an insertion of chromosome 18. Further studies are underway to determine the exact nature of this chromosome abnormality.

Using various techniques, we have identified two patients (patients 17 and 34) for whom molecular studies indicated large deletions, in contrast with the small differences that were noted in the sizes of the normal and abnormal chromosomes by high-resolution G-banding. FISH with chromosome 18–specific paint proved that the abnormal chromosomes contain only chromosome 18–specific material, suggesting a duplication of part of chromosome 18. These patients cannot be used for the phenotypic mapping of the deletion syndromes. Patient 30 will also be excluded, since cytogenetic analysis revealed a large terminal deletion whereas molecular analysis was uninformative for much of the region of interest. In addition, the "normal" chromosome in the patient requires further characterization.

Our finding that some of the terminal deletions identified by routine cytogenetic examinations are actually interstitial deletions contrasts with the results reported by Strathdee et al. (1995), who found that all 26 of their patients with 18q− syndrome had terminal deletions. This is most likely explained by the small sample size and the differences in study populations, although we are aware of some overlap in our patients.

In a group of 35 patients previously reported to have terminal deletions of 18q, we have shown that 5 (14%) contain more-complex cryptic rearrangements. We have also shown that high-resolution karyotype analysis and molecular analysis are both necessary for accurate genotype analysis. Three (9%) of our group of 35 patients would have been incorrectly genotyped by molecular analysis alone, and all 5 subjects would have been incompletely genotyped by high-resolution cytogenetics alone. FISH analysis was required for the detection of the possible paracentric inversion or insertion and of the presence of only chromosome 18 material in the two cases in which translocations were suspected. Correlation of genotype with phenotype will require use of precise genotypic analysis with these complementary techniques. Small duplications of material, similar to those found in two of our patients, would cause trisomy of that chromosomal region. This would be highly detrimental to the phenotype, and the inclusion of such patients would confuse any phenotypic mapping attempts.

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