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Delineation of complex chromosomal rearrangements: evidence for increased complexity

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Abstract There is an assumption of parsimony with regard to the number of chromosomes involved in rearrangements and to the number of breaks within those chromosomes. Highly complex chromosome rearrangements are thought to be relatively rare, with the risk for phenotypic abnormalities increasing as the number of chromosomes and chromosomal breaks involved in the rearrangement increases. We report here five cases of de novo complex chromosome rearrangements, each with a minimum of four breaks. Deletions were found in four cases, and in at least one case, a number of genes or potential genes might have been disrupted. This study highlights the importance of the detailed delineation of complex rearrangements, be-

ginning with high-resolution chromosome analysis, and emphasizes the utility of fluorescence in situ hybridization in combination with the data available from the Human Genome Project as a means to delineate such rearrangements.

Introduction

The majority of constitutional chromosomal rearrangements, which may be either de novo or inherited, are thought to be simple rearrangements, involving one, two, or possibly, three breaks in one or two chromosomes. The occurrence of complex chromosomal rearrangements is believed to be relatively rare. However, a growing number of complex chromosomal rearrangements have been reported (Madan et al. 1997; Peschka et al. 1999; Callen et al. 2002) in which more breaks than expected have been observed. As the rearrangements become more complicated, with more and more breaks, the risk for associated abnormalities increases (Madan et al. 1997).

We report here five cases of de novo complex chromosomal rearrangements, all of which were ascertained postnatally for a variety of phenotypic abnormalities, including developmental delay, mental retardation, dysmorphic features, and/or multiple anomalies. All of the cases were initially analyzed with high-resolution GTG-banding. The cases were then further analyzed by means of fluorescence in situ hybridization (FISH), with whole chromosome paints, centromere-specific probes, subtelomeric probes, and/or bacterial artificial chromosomes (BACs). In two of the five cases, only two chromosomes were involved in the rearrangements, but with a minimum of four breaks in each case. In the remaining three cases, three, four, and six chromosomes were involved, again with a minimum of four breaks. Deletions were found in four of the five cases (at least two of which were cryptic deletions), whereas in at least one case, a gene or genes might have been disrupted by the rearrangement.

The increasing number, availability, and accuracy of BAC probes, largely attributable to the success of the Hu-

Electronic database information: URLs for the data in this article are as follows:

<http://genomics.roswellpark.org/human/overview.html>
<http://genome.ucsc.edu>
<http://www.lanl.gov>

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man Genome Project, has led to the ability to extend the definition of both simple and complex chromosomal rearrangements. This in turn should lead to genotype-phenotype correlations. This study emphasizes the complexity of chromosomal rearrangements and the increasing utility of the combination of high-resolution chromosomes, BACs, and FISH.

Materials and methods

Clinical subjects

Patient 1

This patient was a 26-month-old girl referred for developmental delay and hypotonia. She was the product of an uneventful pregnancy, born by normal vaginal delivery at term to a G₃P₂ mother. Her birth weight was 7 lb 10 oz. Mild respiratory distress was associated with lethargy and hypotonia in the neonatal period. She failed to thrive during the first few months, because of poor sucking and difficulty in swallowing. Her fine and gross motor functions were delayed. She walked at 22 months. She had mild dysmorphism, including bifrontal narrowing of her head, almond-shaped eyes, a depressed nasal bridge, and short hands with tapering fingers. At 4 years of age, she still had hypotonia, and poor gross and fine motor coordination. Her language and memory were age-appropriate. She had impaired sensorimotor function, poor attention span, and poor executive functioning, although she continued to show slow but steady gains in all developmental milestones.

Patient 2

This 2-year-old boy was referred to our laboratory with developmental delay and mild microcephaly. He was the 6 lb 14 oz product of a pregnancy remarkable for maternal lupus and premature rupture of membranes at 36.5 weeks. He had difficulty in maintaining his body temperature in the neonatal period and was noted to have mild hypotonia at 4 months. He sat at 9 or 10 months and walked at 20 months but did not stand without support. He had a short attention span and gaze-avoidance. He had epicanthus tarsalis and persistent fetal fingerpads, and the lateral third of each eyebrow was sparse.

Patient 3

This patient was referred to our laboratory at age 2 with developmental delay and heart abnormalities. He was born at 38 weeks gestation to a G₃P₂ mother, following a pregnancy complicated by a maternal urinary tract infection in the sixth month and oligohydramnios at the time of delivery. His birth weight was less than the 5th percentile. He was noted to have a ventricular septal defect, pulmonary stenosis, and bilateral polycystic kidneys, shown by renal ultrasound after birth. He had borderline low-set ears and micrognathia, but his face was not dysmorphic. The ventricular septal defect was repaired at 9 months of age. He had non-febrile seizures at 12 months, at which age he also first rolled over. At 3 years of age, a cranial computerized tomography scan revealed nodules suggestive of tuberous sclerosis; however, at age 4, follow-up cranial magnetic resonance imaging was more suggestive of cerebral dysplasia or hamartomas. By the age of 6, he was able to sit alone for brief periods of time and had virtually no speech, but he was able to recognize and interact with his parents.

Patient 4

This 17-day-old girl was referred for aniridia and congenital heart defects, including a ventricular septal defect, overriding aorta, and

small pulmonary artery and branches. She was the term product of an uncomplicated pregnancy, with no maternal exposure to smoking, alcohol, or teratogens. Delivery was by emergency Caesarean section because of maternal fever. Birth weight was 5 lb 11 oz and length was 18.5 inches. Her kidneys were normal. A head ultrasound was normal.

Patient 5

This 19-year-old female was ascertained because of multiple anomalies and learning disabilities. She was born at term, after an uneventful pregnancy, and weighed 7 lb 4 oz. She rolled at 5 or 6 months of age, stood at 1 year, and walked at approximately 2 years. Her early medical history was remarkable for hypotonia and bilateral tibial torsion. She had precocious puberty at age 8. She had multiple exostoses or endochondromatosis. Her mental retardation was mild to moderate.

Cytogenetic analysis

Metaphase chromosomes were prepared from peripheral blood lymphocytes or from lymphoblast cultures by standard methods. Lymphocytes from peripheral blood samples were prepared in mitogen-stimulated (phytohemagglutinin and pokeweed) cultures to obtain high-resolution chromosomes (Yunis 1976). The chromosomes were GTG-banded by standard methods (Seabright 1971), and at least 20 metaphase spreads were examined per patient. The resolution for the GTG-banded chromosomes was between 650 and 850 bands.

Lymphoblast cell lines

Lymphoblast cell lines were established according to standard methods by using Marmoset Epstein-Barr virus, phytohemagglutinin, and interleukin-2 (Neitzel 1986). Because of the large number of FISH analyses of patient material, slides from these lymphoblast cell lines were used in the majority of cases to ensure that enough material would be available.

Molecular analysis

All BACs used in these studies were obtained from a Human BAC filter library (RPC1-11) from the Roswell Park Cancer Institute (<http://genomics.roswellpark.org/human/overview.html>). BACs were selected by using the genome browser available from the University of California at Santa Cruz (UCSC; <http://genome.ucsc.edu>). During the course of this study, several different freezes were utilized, including data from October 2000 to April 2003. In addition, for patient 3, LA16 cosmids were used to analyze regions on 16p13.3. The LA16 cosmids were from the chromosome 16 cosmid library from the Los Alamos National Laboratory (<http://www.lanl.gov>). For patient 4, the chromosome 11p13-specific cosmids used were a generous gift from Dr. John Crolla.

BAC DNA was isolated by using the Qiagen Plasmid Purification kit (Qiagen, Valencia, Calif.). The yield of DNA was determined by UV spectrophotometry, and approximately 0.5 µg BAC DNA was used for each FISH-labeling reaction.

Human genome browser

As stated above, BACs were selected by using the genome browser available from UCSC. For all patients, the breakpoints determined by high-resolution chromosome analysis were used to select the cytogenetic bands of interest listed in the browser. Within each band, 4–6 BACs were chosen, by using the unique accession number assigned to each BAC under the full coverage option. Once these BACs had been tested, further BACs distal or proximal to the original selection were chosen, until the analysis was complete.

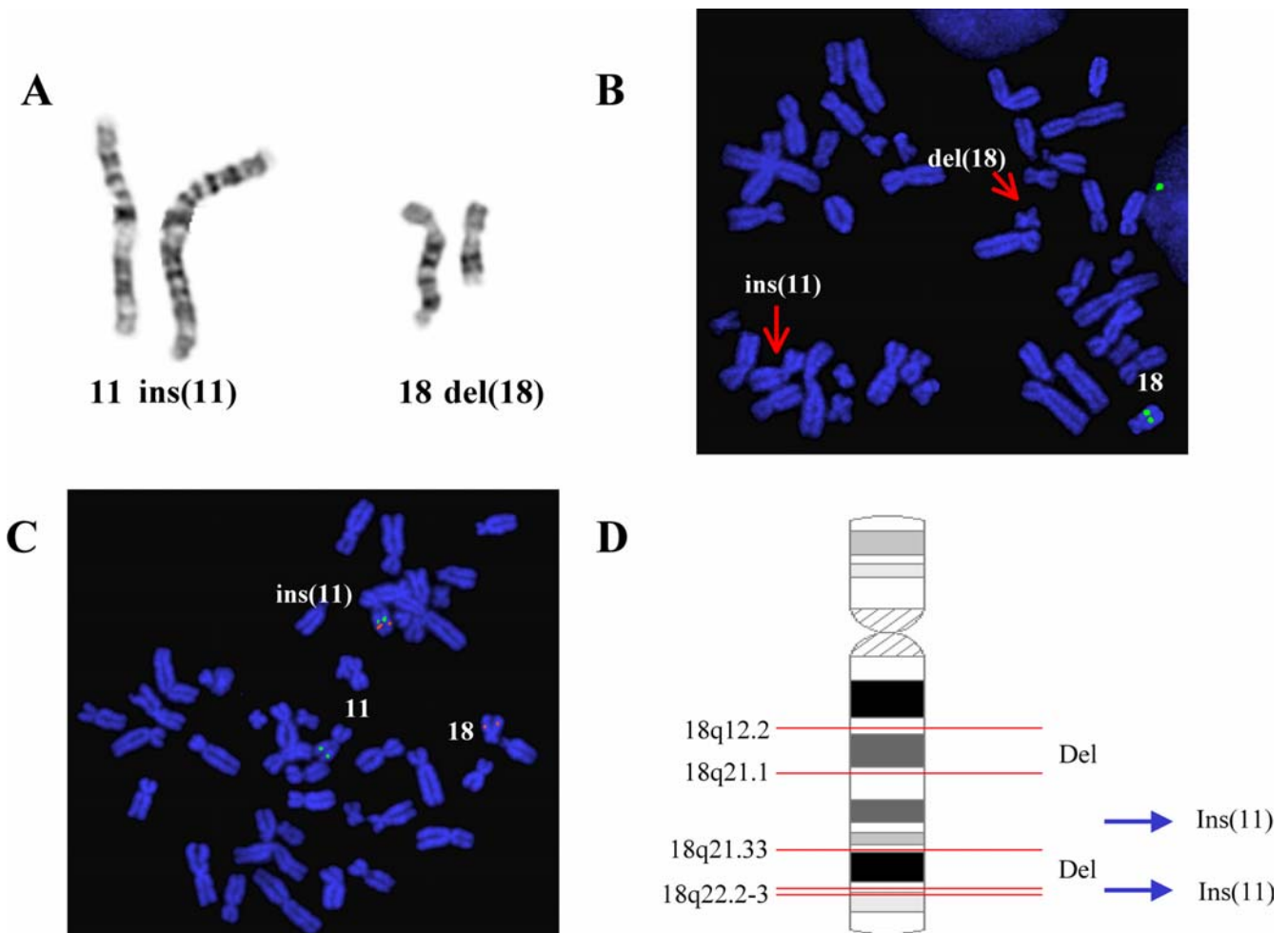


Fig. 1 **A** Partial GTG-banded karyotype of patient 1, showing the normal chromosomes 11 and 18, and ins(11) and del(18). **B** Representative FISH image of the BACs used to delineate the 18q12.2 to 18q21.1 deletion. Only one green signal is seen on the normal chromosome 18 for BAC RP11-19L3 at 18q21.1. **C** FISH analysis of BAC RP11-167K18 at 11q22.3 (green), and BAC RP11-767C4 at 18q21.1 (red). One red signal lies on the normal chromosome 18, and one red signal is found on the inserted chromosome 11, below the green signal for RP11-167K18 (one green signal seen on the normal chromosome 11). This indicates that RP11-167K18 at 11q22.3 is more centromeric than this region of 18q insertion. BAC RP11-767C4 spans the edge of 18q21.1 insertion into chromosome 11. **D** Ideogram of chromosome 18 in patient 1, indicating the breakpoints of the two non-consecutive regions of deletion, and the two non-consecutive regions of insertion into 11q22.3

Once a breakpoint and/or deletion had been delineated by means of the presence or absence of FISH signals or by the splitting of a FISH signal, the number of genes within each breakpoint was determined by means of the data listed under "Genes and Gene Prediction Tracks".

FISH technique

BACs were labeled either indirectly (with digoxigenin, by using the Bionick Labeling kit; Gibco-BRL, USA) or directly (with either Spectrum Orange or Spectrum Green, Nick Translation Labeling kit; Vysis, Downers Grove, Ill.). FISH was performed according to the Oncor protocol (indirect labeling) or the Vysis protocol

(direct labeling), both adapted from standard techniques (Pinkel et al. 1986). FISH with whole chromosome paints, centromere-specific probes, or subtelomeric probes (Vysis) was performed according to the Vysis protocol. Multicolor FISH (M-FISH) was performed by using the SpectraVysion Assay, available from Vysis, following their protocol. FISH images were captured on a Leica DMRB fluorescent microscope and analyzed with Applied Imaging software (Cytovision 2.7). For each probe, at least five metaphase spreads were captured and analyzed.

Results

Patient 1

Initial chromosome analysis performed by another laboratory revealed a karyotype of 46,XX, or 46,XX,del(18)(?q12.2q21.3). Prader-Willi syndrome studies by DNA methylation analysis were normal, as were parental blood karyotypes. High-resolution chromosome analysis in our laboratory revealed a more complex karyotype (Fig. 1A), involving the insertion of part of the long arm of chromosome 18 into the long arm of chromosome 11 and a potential region of deletion in 18q12.2 to 18q12.3 [46,XX,ins(11;18)(q22.2;q12.3q22.1),del(18)(q12.2q12.3)].

The insertion of chromosome 18 into the long arm of chromosome 11 was confirmed by using whole chromo-

some paints for both chromosome 11 and 18 (data not shown). BAC FISH analysis confirmed that there had indeed been a deletion of 18q12.2 to 18q21.1 (Fig. 1B). The proximal breakpoint in 18q12.2 was confirmed by BAC RP11-723J4, for which two signals were seen by FISH, and BAC RP11-49I11 (also at 18q12.2 and immediately adjacent to RP11-723J4), for which only one signal was seen on the normal chromosome 18. The distal breakpoint was established to be in 18q21.1 by means of BAC RP11-701C7 (only one signal at 18q21.1) and the adjacent BAC RP11-767C4 (one signal at 18q21.1 and one signal on 11q; Fig. 1C). BAC RP11-767C4 therefore overlapped the proximal breakpoint of the 18q insertion into 11q and delineated the edge of 18q21.1 deletion. This region of deletion spanned approximately 11 Mb, with the loss of 18 genes.

The distal breakpoint of the 18q insertion was delineated by BAC RP11-851B10 at 18q21.33, which was found on the normal 18q and on the inserted 11, and BAC RP11-575O17 (immediately adjacent to RP11-851B10), which was only found on the normal 18, indicating the presence of a second region of deletion. The distal end of this deletion was delineated by BAC RP11-16B21 (only one signal seen by FISH) at 18q22.2. This second region of deletion, between 18q21.33 and 18q22.2, was approximately 6.5 Mb, with the loss of 15 genes (data not shown).

Interestingly, at this point, a second region of insertion of 18q (18q22.2 to 18q22.3) into chromosome 11 was found. The BAC immediately adjacent to BAC RP11-16B21 (which was deleted) was BAC RP11-704G7 in 18q22.2; this BAC was found on the inserted 11q and on the normal 18q. The distal edge of this 0.8 Mb region of insertion was delineated by BAC RP11-256H12 (in 18q22.2) and the adjacent BAC RP11-47G4 (in 18q22.3); signals for RP11-256H12 were seen on the normal chromosome 18 and the inserted 11, whereas signals for RP11-47G4 were seen on both chromosomes 18. Therefore, there had been two non-consecutive regions of 18q insertion into 11q.

The breakpoints in the long arm of chromosome 11 were more difficult to determine accurately. The edges of the two regions of 18q insertion, as stated above, were delineated by BACs RP11-767C4 (18q21.1), RP11-851B10 (18q21.33), RP11-704G7 (18q22.2), and RP11-256H12 (18q22.2). These four BACs were used to show that the

smaller region of 18q insertion (18q22.2 to 18q22.3) was proximal to the larger region of 18q insertion (18q21.1 to 18q21.33), and that both regions of 18q had inserted into 11q22.3, between BACs RP11-693N9 (above the insertion) and RP11-659O1 (below the insertion). In addition, both insertions of chromosome 18q into the long arm of chromosome 11 were inverted insertions (data not shown). The two 11q22.3 BACs were approximately 0.6 Mb apart according to the UCSC genome browser. However, the insertion breakpoints in 11q22.3 could not be narrowed any further because of the relatively small size of the lymphoblast chromosomes used for the FISH analysis and the necessity of determining the orientation of two small FISH signals in relation to each other.

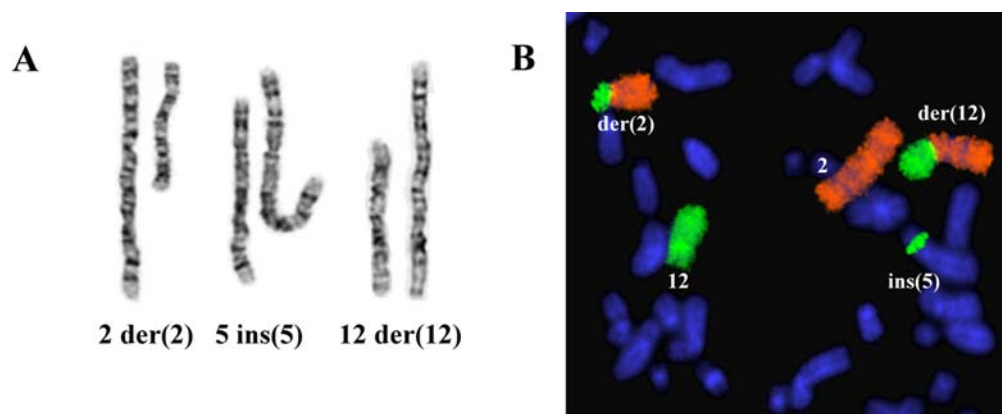
Therefore, in summary, in this patient, there had been a total of five breaks in 18q (Fig. 1D), with two non-consecutive regions of deletion and two non-consecutive regions of insertion, both of which were inserted into 11q22.3.

Patient 2

This patient was referred to our laboratory with the karyotype of 46,XY,t(2;12;5)(p11.1;p11.2;p14)de novo. FISH for the DiGeorge region, with the TUPLE1 probe, was normal. High-resolution chromosome analysis in our laboratory determined that the rearrangement was more complex, involving an insertion of part of the long arm of chromosome 12 into 5p14, rather than a three-way translocation (Fig. 2A). The revised karyotype was designated as follows: 46,XY,t(2;12)(q13;p11.23),ins(5;12)(p14.2;q12q13.13). Chromosome 12 appeared to be broken in at least three places (12p11.23, 12q12, and 12q13.13), whereas the GTG-banding analysis was suggestive of a potential deletion in 12p11.23, 12q13.13, and/or 2q13.

FISH analysis with whole chromosome paints for chromosomes 2 and 12 confirmed the insertion of chromosome 12 into the short arm of chromosome 5 and the presence of chromosome 12 material on chromosome 2, and vice versa (Fig. 2B). FISH analysis of centromere-specific probes for chromosomes 2, 5, and 12 also confirmed the rearrangements (data not shown).

Fig. 2 **A** Partial GTG-banded karyotype of patient 2, showing the normal chromosomes 2, 5, and 12, and the derivative chromosomes 2 and 12, and the ins(5). **B** FISH with whole chromosome paints for chromosomes 2 (red) and 12 (green). The normal chromosome 2 is painted entirely in red, whereas the normal chromosome 12 is painted in green. The translocation between chromosomes 2 and 12 can be seen, as can the insertion of a portion of chromosome 12 into the short arm of chromosome 5



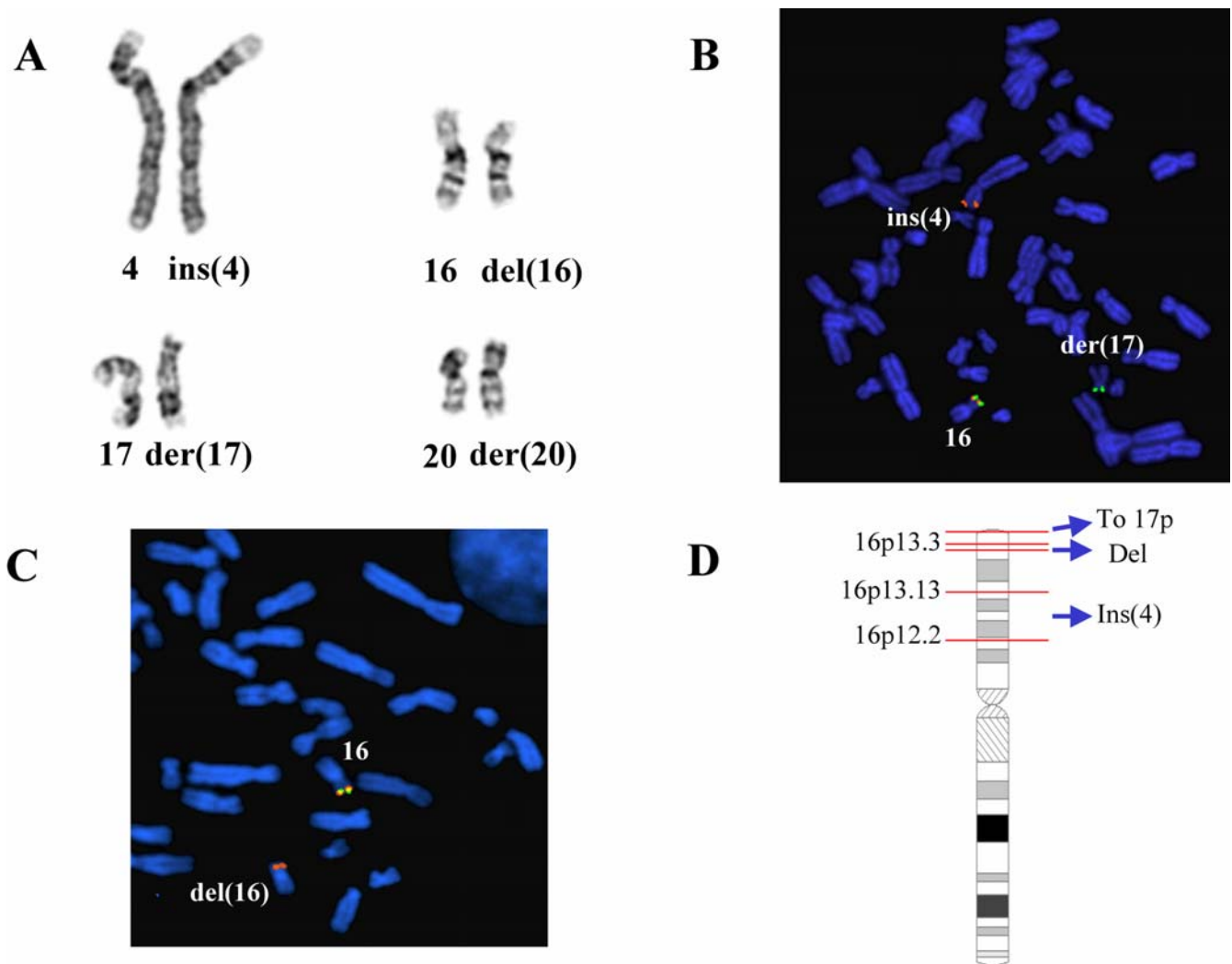


Fig. 3 **A** Partial GTG-banded karyotype for patient 3, showing the normal chromosomes 4, 16, 17, and 20, and the *ins(4)*, *del(16)*, and the derivative chromosomes 17 and 20. **B** FISH analysis of BAC RP11-165M11 at 16p13.13 (*red*), and RP11-616M22 at 16p13.3 (*green*). One *yellow* signal can be seen on the normal chromosome 16 (indicating the presence of both RP11-165M11 and RP11-616M22), whereas one *red* signal for RP11-165M11 can be seen on chromosome 4p, and one *green* signal for RP11-616M22 lies on chromosome 17p. **C** FISH analysis of cosmids LA16-358B7 (*red*) and LA16-380F5 (*green*), both at 16p13.3, showing the deletion of LA16-380F5 on one chromosome 16. A *yellow* signal (both LA16-358B7 and LA16-380F5 present) can be seen on the normal chromosome 16. **D** Ideogram of chromosome 16, indicating the translocation of 16pter to 16p13.3 to 17p, the deletion of approximately 0.8 Mb of 16p13.3, and the insertion of approximately 9 Mb of 16p13.3-16p12.2 into the short arm of chromosome 4

FISH analysis of several BACs was used to delineate the breakpoints among the chromosomes in this rearrangement. The insertion of 12q12 to 12q13.3 into 5p14.1 was delineated by BAC RP11-425I22 (one signal on 12q12 and one signal on the *der(12)*), and the immediately adjacent BAC RP11-367O10, which was present on the *ins(5)* chromosome. The distal edge of the 12q insertion into 5p14.1 was delineated by BAC RP11-340M11 (located in 12q13.3), which was split between the *ins(5)* and *der(12)*

chromosomes. There were no genes present in either of these breakpoint regions, according to the UCSC genome browser.

BACs RP11-349F8 and RP11-261G10, both located in 5p14.1 and adjacent to each other, defined the insertion breakpoint in 5p14.1. RP11-349F8 was above the 12q12 to 12q13.3 insertion, whereas RP11-261G10 was below the insertion. In addition, part of both of these BACs was also seen on the derivative chromosome 2, indicating that there had been an extra break within the short arm of chromosome 5, and within the derivative chromosome 2 (data not shown). There were no genes in the region of 5p14.1 spanned by BACs RP11-349F8 and RP11-261G10.

The translocation between 2q12 and 12p11.23 was delineated by BAC RP11-16L15 (located in 2q12.1), which was found on the *der(2)* chromosome, and the adjacent BAC RP11-1078E8, which was found on the *der(12)* chromosome. In addition, delineation of the breakpoint in the short arm of chromosome 12 confirmed that there was a deletion beginning in band 12p11.22 of approximately 3.6 Mb and extending to the centromeric region of chromosome 12 (data not shown). The deletion was delineated by BAC RP11-498P8 (present on the *der(2)* chromosome and on 12p11.22), and the immediately adjacent BAC

RP11-313F23 (one signal on the normal chromosome 12). Within this 3.6 Mb region, approximately 14 genes were located.

Patient 3

This patient was referred to our laboratory with the karyotype of 46,XY,t(4;16)(p16.1;p13.1). Parental blood samples were normal. High-resolution chromosome analysis (Fig. 3A) in our laboratory suggested that the karyotype was more complex, involving a translocation between chromosomes 4 and 17, with a potential deletion in the short arm of chromosome 16 [46,XY,t(4;17)(p16.3;p13.1),del(16)(p13.11p13.13)]. Subsequent FISH analysis with subtelomeric probes specific for 16p, 17p, and 20q revealed that the subtelomeric region of chromosome 16p had been translocated to 17p, the subtelomeric region of 17p had been translocated to 20q, and the subtelomeric region of 20q had been translocated to chromosome 16p (data not shown).

The breakpoints in 17p and 20q were further analyzed with BAC FISH analysis. The 17p breakpoint was in 17p13.3, between BAC RP11-64J4 (present on 17p and 20q) and the immediately adjacent BAC RP11-147K16 (present on both chromosomes 17). Approximately 3.6 Mb of DNA from the terminal region of chromosome 17p was translocated to the long arm of chromosome 20. The breakpoint in the long arm of chromosome 20 was found in 20q13.33, with BAC RP11-157P1 split between the abnormal chromosome 16p and chromosome 20q (data not shown). Approximately 2 Mb of DNA from 20q was translocated to the short arm of the abnormal chromosome 16.

FISH analysis of several BACs revealed that approximately 9 Mb chromosome 16p (16p13.13 to 16p12.2) was inserted into 4p16.3. The distal edge of the chromosome 16 insertion was determined by BAC RP11-486I11, at 16p13.13, which was present on both chromosomes 16, whereas RP11-166B2, which lay immediately adjacent to RP11-486I11, was present on the normal chromosome 16 and on chromosome 4p. The proximal edge of the chromosome 16p insertion was delineated by BAC RP11-1390J18 (at 16p12.3), present on 16p and 4p, and RP11-338J22 (at 16p12.2), present on the short arm of both chromosomes 16 (data not shown). BACs RP11-1390J18 and RP11-338J22 do not lie immediately adjacent to each other according to the UCSC genome browser, but the BACs between them were unavailable for this study. Consequently, the region of 16p insertion into 4p16.3 may be slightly larger or smaller than the estimated 9 Mb DNA.

The insertion of 16p13.13 to 16p12.2 into 4p16.3 was an inverted insertion and lay between BACs RP11-460I19 (above the insertion) and RP11-572O17 (below the insertion). BAC RP11-460I19 lies approximately 0.5 Mb from the telomere of 4p; the 4p subtelomeric region was still present, as demonstrated by subtelomeric FISH analysis. Interestingly, two BACs, RP11-20I20 and RP11-386I15, which were located immediately between RP11-460I19 and RP11-572O17, had signals on both 4p and 16p, indi-

cating that this small region of 4p16.3 had also been inserted into chromosome 16, probably as a form of reciprocal insertion (data not shown).

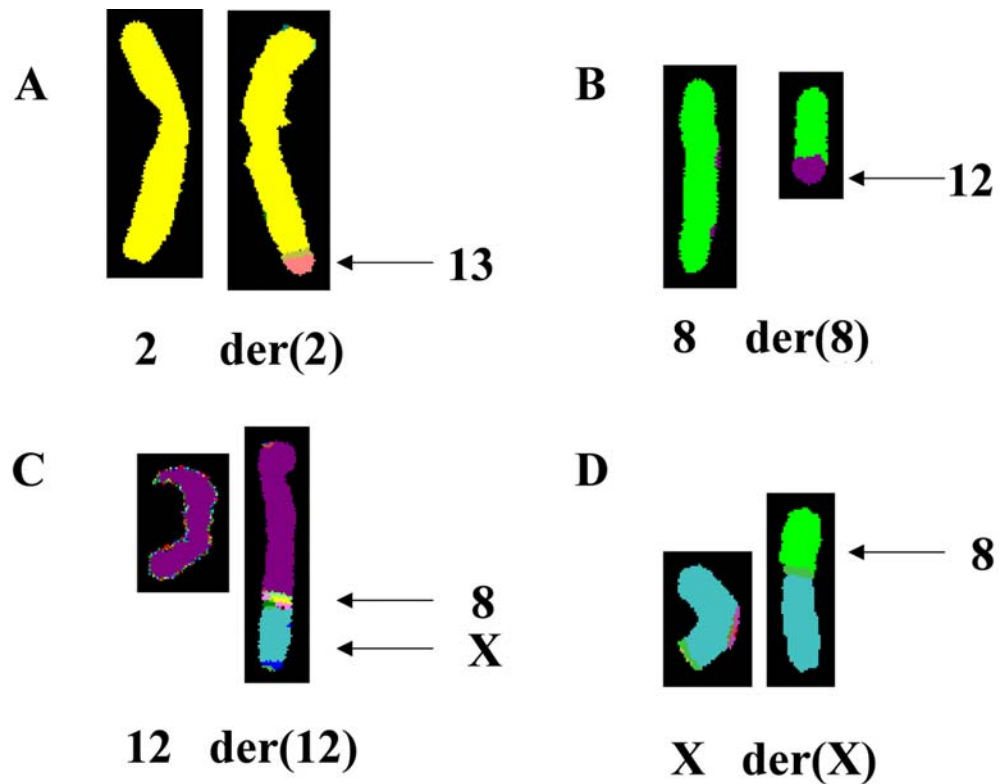
As stated above, the region of 20qter to 20q13.33 was present on the abnormal chromosome 16 at 16p13.3, whereas the region of 16p13.3 to 16pter was present on 17p (approximately 1.3 Mb). This breakpoint in 16p13.3 was delineated by BAC RP11-616M22 (Fig. 3B), with signals seen on the normal chromosome 16 and on the derivative chromosome 17. Cosmid LA16-358B7, which was proximally adjacent to BAC RP11-616M22, was present on both chromosomes 16. Cosmid LA16-399E4, which was proximally adjacent to LA16-358B7, was deleted (Fig. 3C). The proximal edge of this deletion was delineated by cosmid LA16-439A6. This cryptic deletion was about 0.8 Mb, with the loss of approximately 50 genes. BAC RP11-304L19, which is approximately 0.14 Mb proximal to cosmid LA16-439A6 in 16p13.3, was present on both chromosomes 16p and chromosome 4p, indicating a second small region of insertion of 16p13.3 into 4p. This cryptic insertion was not defined in more detail, although FISH with a plasmid artificial chromosome probe specific for the tuberous sclerosis gene (TSC2) at 16p13.3 revealed that this gene was present on chromosome 4p and on the normal chromosome 16. The TSC2 gene overlaps BAC RP11-304L19 and is closely linked to PKD1 (the gene for autosomal dominant polycystic kidney disease), which is also located in the region of BAC RP11-304L19. BAC RP11-657D15 (16p13.3) was immediately adjacent to RP11-304L19 and was present on both chromosomes 16.

In summary, in this patient, there had been a minimum of five breaks in 16p (Fig. 3D), plus one break in 17p, one break in 20q, and a minimum of two breaks in 4p.

Patient 4

High-resolution chromosome analysis in this patient revealed an extremely complex karyotype, involving chromosomes 2, 8, 11, 12, and 13, and the X chromosome [46,X,der(X)(8qter→8q?21.2::Xp?21→Xqter),t(2;13)(2pter→2q37.2::13q31→13qter),der(8)(8pter→8q11.23::12q24.31→12qter),del(11)(11pter→11p14.2::11p11.2→11qter),der(12)(12pter→12q15::12q24.31→12q15::8q?→8q?::Xp?21→Xpter)]. Parental blood samples were normal. FISH with a whole chromosome paint for chromosome 11 revealed that there was no other chromosomal material attached to the deleted chromosome 11, nor had the missing portion of 11p been translocated to another chromosome (data not shown). The deletion in 11p was confirmed with cosmid probes B2.1, P60, FAT5, and FO2121, which are specific to 11p13 (Crolla and van Heyningen 2002). Only one signal for each probe was seen on the normal chromosome 11 (data not shown). Multicolor FISH (Fig. 4) and FISH with whole chromosome paints for chromosomes 2, 8, 12, and 13 and the X chromosome confirmed the reciprocal rearrangement between one chromosome 2 and one chromosome 13, and the possibly balanced rearrangement between one X

Fig. 4 **A** Representative M-FISH image of the normal chromosome 2 (yellow) and the derivative chromosome 2 (chromosome 13 material in brown) from patient 4. The reciprocal derivative chromosome 13 is not shown. **B** Representative M-FISH image of the normal chromosome 8 (green) and the derivative chromosome 8 (chromosome 12 material in purple). **C** Representative M-FISH image of the normal chromosome 12 (purple) and the derivative chromosome 12 (X chromosome material in blue and chromosome 8 material in green). **D** Representative M-FISH image of the normal X chromosome (blue) and the derivative X chromosome (chromosome 8 material in green)



chromosome, one chromosome 8, and one chromosome 12. However, lack of material prevented further characterization of the breakpoints in this patient with BACs.

Patient 5

This patient was referred with the karyotype of 46,XX,t(6;10)(q21;q25.2). FISH with probes specific for the Prader-Willi/Angelman and DiGeorge (TUPLE1) region was normal. The rearrangement was de novo. High-resolution chromosome analysis in our laboratory confirmed the translocation between chromosomes 6 and 10 (Fig. 5A) and also revealed a pericentric inversion within the der(6) chromosome, with breakpoints assigned to 6p23 and 6q22.2. FISH analysis with whole chromosome paints for chromosomes 6 and 10 confirmed the translocation. The inversion was initially confirmed with subtelomeric probes for 6p, 6q, and 10q. The subtelomeric probe for 10q was located on the der(6) chromosome, whereas the subtelomeric probe for 6p was located on the opposite end of the der(6) chromosome. The subtelomeric probe for 6q was found on the der(10) chromosome (data not shown).

Since the GTG-banded analysis was suggestive of a deletion in the region 10q26.2 to 10q26.3, this area was used initially to select BACs. No deletion was found. BAC RP11-391M7 at 10q26.13 was split between the der(10) and the der(6) chromosomes. The breakpoint in 6q in the der(6) chromosome was delineated by BAC RP11-544L8 (in 6q21), which was split between the der(6) and der(10) chromosomes (Fig. 5B). The breakpoints of

the pericentric inversion in the der(6) were delineated by BAC RP11-346C16 (in 6q21), which was split between the two arms of the der(6) chromosome, and BAC RP11-421M1 (in 6p24.2), which was also split between the two arms of the der(6) chromosome.

Therefore, in summary, there had been a minimum of four breaks in this patient's chromosomes. No deletion was found at any of the breakpoint regions; in addition, approximately 14 genes spanned the breakpoints (according to the UCSC genome browser), which may or may not have been disrupted by the rearrangements.

Discussion

We report here five cases of complex chromosome rearrangements, each with a minimum of four breaks within the chromosomes involved. All of the cases were ascertained postnatally because of phenotypic abnormalities. Two of the five cases had complex chromosome rearrangements involving two chromosomes (one insertion/deletion and one translocation/inversion), whereas the remaining cases had three, four, and six chromosomes involved in the rearrangement (translocations and insertions). Cryptic deletions were found in four cases, and in at least one case, a number of genes or potential genes may have been disrupted.

Patient 1 was referred to our laboratory with a potential deletion of the long arm of chromosome 18. High-resolution chromosome analysis revealed that part of the long arm of chromosome 18 had been inserted into the long

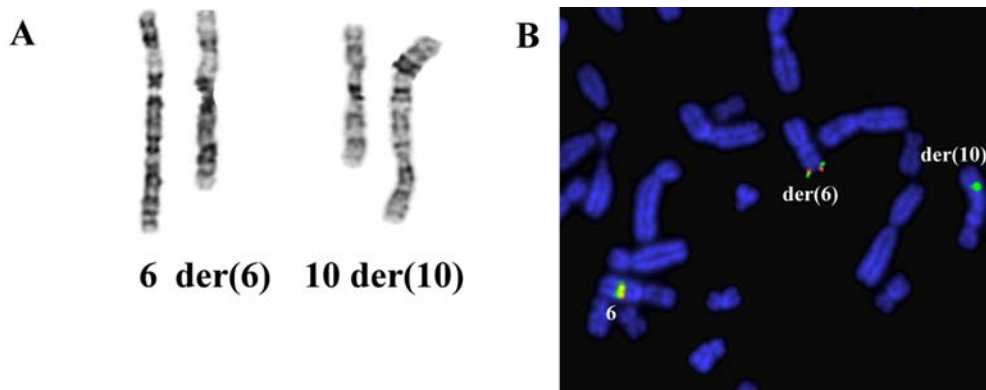


Fig. 5 **A** Partial GTG-banded karyotype of patient 5, showing the normal chromosomes 6 and 10, and the derivative chromosomes 6 and 10. **B** FISH analysis of BACs RP11-544L8 (*green*), and RP11-437J19 (*red*), both at 6q21. One *yellow* signal can be seen on the normal chromosome 6. In addition, a *yellow* signal is found on the “short” arm of the inverted chromosome 6, indicating the presence of both BACs RP11-544L8 and RP11-437J19. A *green* signal (RP11-544L8) can also be seen on the derivative chromosome 10, indicating that RP11-544L8 is split between the derivative chromosomes 6 and 10

arm of chromosome 11. FISH analysis of several BACs confirmed the deletion in chromosome 18 (18q12.2 to 18q21.1) and defined a second non-consecutive region of deletion (18q21.33 to 18q22.2). The amounts of DNA deleted were, respectively, 11 Mb and 6.5 Mb. Within these regions, 18 and 15 genes, respectively, were deleted. There were also two non-consecutive regions of insertion of chromosome 18 into 11q22.3 (18q21.1 to 18q21.33, and 18q22.2 to 18q22.3). Therefore, a total of five breaks had occurred within one chromosome 18.

Deletions of the long arm of chromosome 18 occur in approximately 1 in 40,000 live born infants (Cody et al. 1999), with even quite large deletions in this region of the genome being compatible with life. Cody et al. (1999) studied 42 individuals with deletions of chromosome 18q, comparing phenotypic findings among these individuals, and suggesting that deletions of 18q may represent a contiguous gene deletion syndrome. Our patient had two non-contiguous interstitial deletions, with the loss of approximately 18 Mb DNA in total. Some patients with 18q deletions may have lost up to 36 Mb DNA (Cody et al. 1999), which may explain the relatively mild phenotype of this patient compared with other 18q deletion patients.

Patient 2, referred to our laboratory with a complex rearrangement involving chromosomes 2, 5, and 12 was found to have at least six breaks, four of which were in chromosome 12. In addition, a cryptic deletion of about 3.6 Mb was found beginning in band 12p11.22, extending to the centromere, and involving the loss of approximately 14 genes. There have been a few reports in the literature of interstitial deletions of chromosome 12p (Glaser et al. 2003), with phenotypes including mental retardation, psychomotor retardation, and facial dysmorphism. Our patient had some psychomotor retardation and mild facial dysmorphism.

There were no genes present in the breakpoint regions of 2q12.1, 5p14.1, 12q12, or 12q13.3 (according to the UCSC genome browser), whereas the delineation of the breakpoints revealed an even higher degree of complexity than expected. A small insertion of part of 5p14.1 into the derivative chromosome 2 was seen when the insertion of 12q into chromosome 5 was being delineated. Despite this additional level of complexity and the additional chromosomal breaks, the phenotype in this patient appeared to be relatively mild, suggesting that increasing complexity in chromosomal rearrangements may not always have an impact on patient phenotype, or that breakage in some regions of the genome results in fewer phenotypic consequences.

Patient 3 was referred to our laboratory with the karyotype of 46,XY,t(4;16)(p16.1;p13.1). Subsequent FISH analysis of several BACs revealed a far more complex karyotype, with subtelomeric rearrangements involving 16p, 17p, and 20q, an insertion of approximately 9 Mb of 16p (16p13.13 to 16p12.2) into 4p16.3, a small reciprocal insertion of 4p16.3 material into 16p, a second small insertion of 16p13.3 into 4p, and a cryptic deletion of 0.8 Mb in 16p13.3. There have been several reports in the literature of deletions in or rearrangements involving 16p13.3, which is a highly gene-rich area (Brook-Carter et al. 1994; Brown et al. 2000; Eussen et al. 2000; Horsley et al. 2001).

The 0.8-Mb cryptic deletion in 16p13.3 resulted in the loss of approximately 50 genes. In addition, part of BAC RP11-304L19 in 16p13.3 (which is within approximately 380 kb of the deletion) was inserted into chromosome 4, as was the gene for TSC2 (located in the region of RP11-304L19). The gene for autosomal dominant PKD1 is found in the region of RP11-304L19, and this patient was reported as being affected by polycystic kidney disease. The function of the PKD1 gene and/or the TSC2 gene may have been disrupted as a result of a position effect, which has been reported in rearrangements involving chromosome 11 (Fantes et al. 1995), chromosome 16 (Barbour et al. 2000), and chromosome 22 (Sutherland et al. 1996), for example. However, because of the high complexity of the karyotype of this patient, the assignment of a single gene disruption or deletion as the cause of the phenotype of the patient is difficult at this stage; more than one gene probably contributes to the phenotypic findings, and posi-

tion effects may influence the genes involved. In summary, therefore, in this patient, there had been at least five breaks within a small region of 16p13.3. There had also been one break in 17p and 20q, and at least two breaks in 4p16.3.

The most complex rearrangement was seen in patient 4, involving six chromosomes (chromosomes 2, 8, 11, 12, and 13, and the X chromosome). There have been a few reports in the literature of live-born patients with highly complex chromosomal rearrangements involving up to four chromosomes, some of which have been familial (Rothlisberger et al. 1999) and some of which have been de novo (Kaiser-Rogers et al. 2000). In our patient, in addition to the translocations observed, a deletion in 11p was seen; this deletion is associated with aniridia-Wilms' tumor. The phenotype of this 17-day-old girl included a congenital heart defect (ventricular septal defect, overriding aorta, and small pulmonary artery and branches), and aniridia. Unfortunately, because of lack of material, the breakpoints in this patient could not be investigated further.

In contrast to the other cases, no deletion was found in patient 5. This patient, referred with the karyotype of 46,XX,t(6;10)(q21;q25.2), was again shown to have a more complex rearrangement than originally anticipated. In this patient, at least four breaks had led to the rearrangement between chromosomes 6 and 10, which also involved a pericentric inversion in the derivative chromosome 6. Band 6q21 has been reported as being involved relatively frequently in pericentric inversions (Kleczkowska et al. 1987); our patient had two breakpoints within this band. In addition, at least 14 genes might have been disrupted at the various breakpoints delineated in the rearrangement. This patient was referred with the phenotype of mild to moderate retardation, an early history of hypotonia and bilateral tibial torsion, and multiple exostoses. It is difficult to assign the impact of any of the potential gene disruptions to the phenotype of our patient; further investigation is needed into the function of these genes and their suspected disruption.

The assumption of parsimony with regard to chromosomal rearrangements may be summarized by Occam's Razor, in which 'plurality should not be posited without necessity'. In the five cases discussed here, the original karyotypes were suggestive of simpler chromosomal rearrangements, and the 'plurality' was ultimately determined to be the case with further FISH analysis of BACs. As rearrangements become more complicated, with more and more breaks, the risk for associated abnormalities is presumed to increase. In patients 2 and 3, an even greater degree of complexity was found when the fine-tuning of the delineation of the breakpoints was completed; patient 3 was indeed severely compromised, although patient 2 had a relatively mild phenotype.

Both deletions (ranging in size from 0.8 Mb to 11 Mb), and potential gene disruptions were seen in the patients described here. Deletions have been theorized as a cause of phenotypic abnormalities in patients with apparently "balanced" rearrangements (Kumar et al. 1998; Astbury et

al. 2004), whereas gene disruptions are a well-known cause of phenotypic abnormalities. However, to assess fully the impact of any or all of the potential gene disruptions in these patients, functional studies of all the genes will be needed.

In summary, this study highlights the importance of the delineation of complex chromosomal rearrangements by means of high-resolution GTG-banding, FISH, BACs, and the data available from the Human Genome project. As demonstrated by these five cases, complex rearrangements may prove even more complex than originally anticipated, findings that may eventually provide phenotypic answers for the patients and their families.

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