Am. J. Hum. Genet. 50:914–923, 1992

The Presence of Interstitial Telomeric Sequences in Constitutional Chromosome Abnormalities

Vicki Murtif Park,[1] Karen M. Gustashaw, and Theresa M. Wathen

Genetics Center, Department of Pediatrics, Case Western Reserve University, Cleveland

Summary

We describe a novel chromosome structure in which telomeric sequences are present interstitially, at the apparent breakpoint junctions of structurally abnormal chromosomes. In the linear chromosomes with interstitial telomeric sequences, there were three sites of hybridization of the telomere consensus sequence within each derived chromosome: one at each terminus and one at the breakpoint junction. Telomeric sequences also were observed within a ring chromosome. The rearrangements examined were constitutional chromosome abnormalities with a breakpoint assigned to a terminal band. In each case (with the exception of the ring chromosome), an acentric segment of one chromosome was joined to the terminus of an apparently intact recipient chromosome. One case exhibited apparent instability of the chromosome rearrangement, resulting in somatic mosaicism. The rearrangements described here differ from the telomeric associations observed in certain tumors, which appear to represent end-to-end fusion of two or more intact chromosomes. The observed interstitial telomeric sequences appear to represent nonfunctional chromosomal elements, analogous to the inactivated centromeres observed in dicentric chromosomes.

Introduction

Telomeres play a critical role in maintaining chromosomes as discrete functional units. The telomere performs two key functions (for review, see Zakian 1989; Blackburn 1991). First, it provides a "cap" for the end of the chromosome, thus preventing degradation of the DNA and recombinogenic activity between chromosomes. In addition, the telomere provides for complete replication of chromosome ends during the process of discontinuous strand synthesis. The fundamental importance of these functions led to the assumption that stable, linear chromosomes contain two telomeres, one at each end of the molecule (Muller 1939; Holmquist and Dancis 1979). This expectation is currently applied to the practice of clinical cytogenetics in the interpretation of structural abnormalities. For example, in a phenotypically normal carrier of a balanced translocation, the rearrangement is assumed to be reciprocal, even in a rearrangement that appears to be a single segment transfer. A breakpoint in the terminal band of the recipient chromosome is inferred, even when no reciprocal transfer of chromatin is apparent at the microscopic level. This assumption is necessary if the rearranged chromosomes are to conform to the standard configuration, with a telomere at each terminus.

Recent work has provided a more detailed characterization of telomere structure and function. Moyzis et al. (1988) identified and characterized a human telomere consensus sequence, $(TTAGGG)_n$, and suggested that this sequence is a functional human telomere. This hexanucleotide sequence was found to be present at the ends of all human chromosomes, and strong evolutionary conservation was observed among vertebrates (Meyne et al. 1989). Work in lower eukaryotes has led to the identification of telomere-binding proteins, which probably are key elements in providing stability to chromosome ends (Gray et al.

Received August 21, 1991; revision received December 20, 1991.

1. Current address and address for correspondence: Vicki M. Park, Ph.D., Room 523, 711 Jefferson Avenue, University of Tennessee, Memphis, TN 38163.

Address for reprints: Karen M. Gustashaw, M.S., Genetics Center, 11001 Cedar Avenue, Suite 510, Cleveland, OH 44106.

0002-9297/92/5005-0005$02.00

1991). The process of telomere replication is carried out by a separate protein moiety, telomerase. This novel ribonucleoprotein catalyzes the addition of a species-specific telomere repeat by priming off of G-rich sequences. Telomerase was first described in the ciliated protozoan, Tetrahymena (Greider and Blackburn 1987, 1989), and a similar activity was identified in the human cell line, HeLa (Morin 1989). All of these studies suggest that the telomere consensus sequence plays a critical role in the function of chromosome ends.

In situ hybridization of the telomere consensus sequence to metaphase chromosomes provides a method to examine the distribution of telomeric sequences in structurally rearranged chromosomes. We used this approach to analyze constitutional structural abnormalities containing a terminal breakpoint. Several of the rearrangements examined complied with the expected configuration of one telomere at each terminus, since there was one site of hybridization at each end of the structurally abnormal chromosomes. No chromosomes were found to be lacking a terminal telomere. Unexpectedly, five different structurally abnormal chromosomes were found to contain telomeric sequences at breakpoint junctions. The abnormal linear chromosomes had a telomere at each end, in addition to the interstitial telomeric sequences at the breakpoint junction. A ring chromosome exhibited a single set of telomeric sequences. Our observations suggest a loss of telomere function in this novel class of chromosome rearrangements.

Subjects and Methods

Subjects

Case 1.—In the newborn period, this patient was noted to be extremely hypotonic and to exhibit poor feeding. Two peripheral blood specimens were collected, at 8 and 16 d of age. A skin biopsy was obtained at 15 d of age. Cytogenetic studies of all three specimens revealed unbalanced karyotypes with monosomy 15pter→q13 (table 1 and fig. 1). Mosaicism consistent with a jumping translocation was observed. Three cell lines were identified in a total of 250 cells examined. A fourth rearrangement, 45,XX, − 15, − 20, + der(20)t(15;20)(q13;qter), was observed in a single cell. Cytogenetic and molecular studies and the clinical findings are consistent with a diagnosis of Prader-Willi syndrome (Park et al. 1991). Both parents are chromosomally normal.

Case 2.—This patient was referred for cytogenetic analysis as an adolescent with a diagnosis of Prader-Willi syndrome. Analysis revealed an unbalanced karyotype with monosomy 15pter→q13 (table 1 and fig 1). The 112 cells examined originated from a single peripheral blood specimen.

Case 3.—This patient was a newborn with multiple congenital anomalies, including cardiac anomalies, talipes equinovarus, dysmorphic facies, and posteriorly rotated ears. Cytogenetic analysis revealed an unbalanced karyotype with extra material on the long arm of chromosome 2 (table 1 and fig. 1). Chromosome painting was used to establish that the extra material originated from chromosome 17 (data not shown). Since two normal copies of chromosome 17 are present, the patient possesses a partial trisomy of 17q23→qter. Both parents are chromosomally normal.

Case 4.—This patient exhibited severe mental retardation and dysmorphic facies and walked with an unusual gait. Chromosomes were first studied elsewhere at 1 year of age; the patient was referred for follow-up cytogenetic studies at the age of 15 years. Three cell lines were observed (table 1). The predominant cell line (73%) contained a ring chromosome consisting of all or most of a chromosome 18 plus a small amount of material that may be from the pericentromeric region of chromosome 9 (fig. 1). This cell line contains one normal copy of chromosome 18 and two normal copies of chromosome 9. In several cells, variants of this karyotype were noted, with either two copies of the ring chromosome or a double-sized ring. This is typical behavior for a ring chromosome. Both C-banding for centromeric heterochromatin and in situ hybridization with a centromeric cocktail probe indicated that the ring chromosome contains a single centromere (data not shown). The ring chromosome presumably originated from a linear derived chromosome that was observed in 12% of the cells examined. In place of the ring chromosome, the cells contained a structurally abnormal chromosome consisting of all or most of a chromosome 18 joined to the long arm of chromosome 9 and, possibly, the proximal short arm of chromosome 9. In addition, 15% of the cells examined exhibited monosomy 18, presumably the result of loss of the ring chromosome. Both parents were reported to be chromosomally normal.

Case 5.—This case involved a familial chromosome abnormality with stalks and satellites present on the terminus of the long arm of chromosome 1 (table 1 and fig. 1). The abnormality is present in a man and

Table 1

Karyotype Designations

Case	Karyotype(s)[a]
1	45,XX, − 15, − 19, + der(19)t(15;19)(q13;pter)/ 45,XX, − 8, − 15, + der(8)t(8;15)(pter;q13)/ 45,XX, − 1, − 15, + der(1)t(1;15)(qter;q13)de novo
2	45,XY, − 5, − 15, + der(5)t(5;15)(qter;q13)
3	46,XX, − 2, + der(2)t(2;17)(qter;q23)de novo
4	45,XY, − 18/46,XY,der(18)t(9;18)(qter→?9p13::18p11.3→18qter)/ 46,XY, r der(18)(?9p11→?9p13::18p11.3→18q23)de novo
5	46,XX or XY,1qs
6	46,XY,2p + de novo
7	46,XY,20p + de novo
8	46,XX or XY,t(1;10)(p36.22;q26.3)
9	46,XY,t(1;11)(p36.3;q13.1)
10	46,XY,del(2)(q37.2)de novo
11	46,XX,del(13)(q33)de novo
12	46,XY,del(14)(q32.3)de novo

[a] In cases where interstitial telomeric sequences were detected, the terminal breakpoint is designated as "pter" or "qter," rather than as a band designation.

in his daughter, where it was first detected after routine amniocentesis performed for advanced maternal age. The 1qs chromosome exhibited positive silver staining for nucleolar organizer regions, indicating that the extra material did originate from the short arm of an acrocentric chromosome. C-banding was positive both at the chromosome 1 centromere and in the distal long arm region corresponding to band p11 of an acrocentric chromosome. In addition, the abnormal chromosome stained negatively with DAPI/distamycin A, indicating that the extra material is unlikely to be derived from a chromosome 15 (results of additional staining are not shown). In both individuals, all of the acrocentric chromosomes appeared to have normal morphology. Therefore, it is unlikely that the 1qs chromosome is one derivative of a balanced translocation in either individual studied. Both individuals exhibit a familial trait of spongy nevus of the mouth but are otherwise healthy.

Case 6.—This patient was a newborn with multiple dysmorphic features, including small palpebral fissures, flat nasal bridge, bifid uvula, apparently lowset ears, and a unilateral single palmar crease. Cytogenetic analysis revealed an unbalanced karyotype with extra material on the short arm of chromosome 2 (table 1 and fig. 1). The terminal band of chromosome 2 (2p25.3) appears to be present, with additional material of undetermined origin joined distally. Chromosome painting with a chromosome 2–specific probe was used to demonstrate that the extra material is from a chromosome other than 2 and that, therefore, it does not represent a duplication (data not shown). Both parents are chromosomally normal.

Case 7.—This chromosome abnormality was first detected after amniocentesis for fetal anomalies observed by ultrasound (apparent cystic hygroma and diaphragmatic hernia). Cytogenetic analysis revealed the presence of an unbalanced fetal karyotype with extra material joined to the end of the short arm of chromosome 20 (table 1 and fig. 1). The origin of the additional material is unknown. Elective termination was performed. Both parents are chromosomally normal.

Case 8.—This case involved a familial balanced translocation between chromosomes 1 and 10. The rearrangement was first detected in the husband of a couple referred for multiple spontaneous abortions. Subsequently, the couple delivered a daughter who is also a balanced carrier. The chromosome 10 breakpoint is in the terminal band of the long arm.

Case 9.—This case involved a familial balanced translocation between chromosomes 1 and 11. The rearrangement is present in a man and in his daughter, who was born with multiple congenital anomalies. The child carries the balanced form of the paternal translocation. In addition, she carries an unbalanced form of a maternal translocation between chromosomes 11 and 16. The child's karyotype is 46,XX, − 11, + der(11)t(11;16)(q25;p12.2)mat,t(1;11)(p36.3;q13.1)pat. The chromosome 1 breakpoint

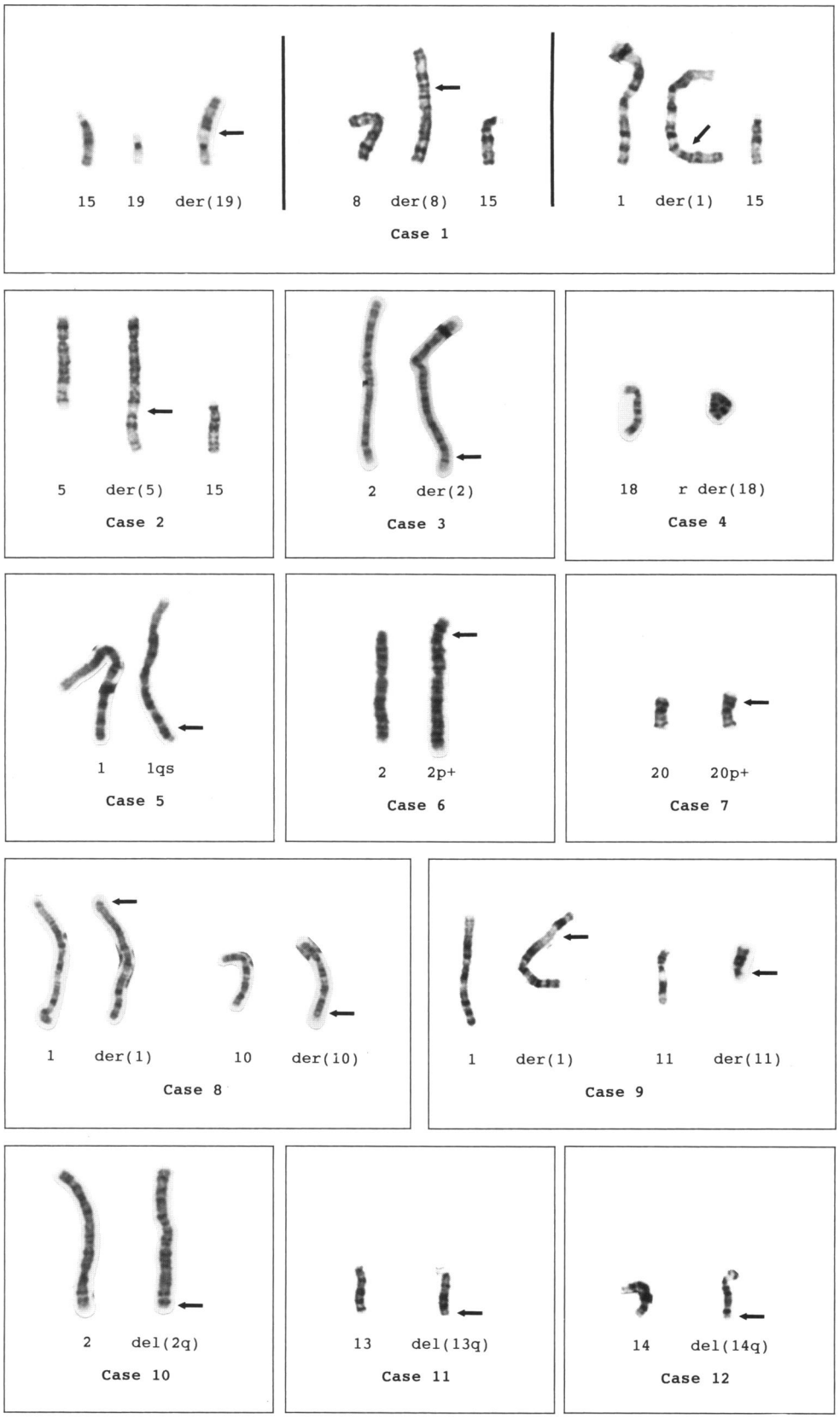


Figure 1 Partial karyotypes, showing Giemsa-banded preparations of the structurally abnormal chromosomes described in cases 1–12. In each panel, the abnormal chromosome is shown to the right of its normal homologue. An arrow indicates the position of the breakpoint in each abnormal chromosome.

of the paternal translocation is in the terminal band of the short arm.

Case 10.—This patient was referred for cytogenetic analysis at 2 years of age, because of developmental delay. The study revealed a small terminal deletion of the long arm of chromosome 2 (table 1 and fig. 1). Both parents are chromosomally normal.

Case 11.—This patient was referred for cytogenetic analysis in the newborn period, because of micrognathia and intrauterine growth retardation. The study revealed a small, terminal deletion of chromosome 13 (table 1 and fig. 1). Both parents are chromosomally normal.

Case 12.—This patient was referred for cytogenetic analysis at 6 mo of age, because of dysmorphic facies. The study revealed a small, terminal deletion of chromosome 14 (table 1 and fig. 1). Both parents are chromosomally normal.

Methods

Cytogenetic analyses were performed according to standard procedures. Giemsa-banding was used for routine analysis of metaphase preparations.

In situ hybridizations with the telomere probe were carried out according to procedures published by others (Moyzis et al. 1988; Meyne et al. 1989). In brief, two synthetic oligonucleotides corresponding to complementary strands of the consensus repeat, $(GGGTTA)_7$ and $(TAACCC)_7$, were synthesized on an Applied Biosystems DNA synthesizer. The oligonucleotides were labeled using biotin-16-dUTP and terminal transferase (Boehringer Mannheim). Freshly prepared slides were pretreated with RNase A (100 μg/ml) and then were denatured in 70% formamide (Fluka) in 2 × SSC (1 × SSC = 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) at 70°C for 2 min. After dehydration of the slides, the labeled probe was applied at a concentration of 0.5 μg/ml in 2 × SSC, 30% formamide with salmon sperm DNA (100 μg/ml) added as carrier. Slides were hybridized overnight at 37°C and then were washed at 42°C in 2 × SSC, 30% formamide for 20 min, followed by two changes of 2 × SSC at 37°C for 4 min each. Slides were stored in PN buffer (0.1 M sodium phosphate, pH 8.0, 0.1% Nonidet P-40) until further analysis (Pinkel 1988).

Hybridization was detected by using fluorescein avidin and biotinylated anti-avidin antibody (both from Vector Laboratories). One or two rounds of amplification were performed in different experiments. Chromosomes were counterstained with propidium iodide (Sigma) and DAPI (Sigma) and were visualized by using a Leitz Diaplan microscope with appropriate filters. Metaphase spreads were photographed by using Fuji 400 print film with exposure times of approximately 45 s for fluorescein. The fluorescein signal and propidium iodide counterstain were visualized simultaneously, and the DAPI stain, which produced a faint banding pattern, was observed with a separate filter.

Centromere probes were purchased from Oncor and used according to instructions. Chromosome painting was performed according to procedures provided by the supplier of the probes (Imagenetics). The chromosome 17–specific probe was labeled directly with a fluorescent compound, spectrum orange; thus, indirect detection and amplification were unnecessary.

Results

The reported cases were chosen on the basis of possessing either a terminal breakpoint or a terminal deletion. Cases 1–9 exhibit a breakpoint in a terminal band and were analyzed for the presence or absence of interstitial telomeric sequences. Cases 10–12 exhibit terminal deletions and were examined for the possible absence of a terminal telomere. Initially, all specimens were analyzed using Giemsa-banded chromosome preparations. Additional staining procedures were performed as described in the case histories. Karyotypic designations are listed in table 1. Standard metaphase preparations were used for in situ hybridizations with biotin-labeled probes specific for the telomere consensus sequence. In normal cells, these probes hybridize only to chromosome ends (Moyzis et al. 1988). Since the efficiency of hybridization is less than 100%, a signal is not observed at every chromosome end in a given cell. Furthermore, signal intensity is variable and is not necessarily indicative of the amount of telomeric DNA at a given site of hybridization.

Presence of Interstitial Telomeres

Case 1 exhibited a jumping translocation. This type of chromosome abnormality has been reported in several patients with Prader-Willi syndrome (for review, see Rivera et al. 1990). Mosaicism appears to be a consequence of chromosome instability. In this patient, three different cell lines were observed, and mosaicism was documented in both lymphocytes and fibroblasts. All cell lines exhibited monosomy of the short arm and proximal long arm of chromosome 15 (15pter→q13). The chromosome 15 long arm segment (15q13→qter) was involved in a terminal transloca-

tion with either chromosome 19 (pter), chromosome 8 (pter), or chromosome 1 (qter) (table 1 and fig. 1). In the rearranged chromosomes, in situ hybridization with the telomere probe identified three distinct sites of hybridization. In addition to two terminal telomeres, telomeric sequences were detected interstitially in the derived chromosome 19 (fig. 2*a*), the derived chromosome 8, and the derived chromosome 1 (data not shown). Each set of interstitial telomeric sequences was localized to the region of the breakpoint junction (compare fig. 2*b*). The simplest interpretation of these findings is that the broken segment of chromosome 15 (15q13→qter) is attached to the terminus of each recipient chromosome, thus producing derived chromosomes with three sets of telomeric sequences, two terminal and one interstitial.

In case 2, a similar type of rearrangement was observed, but no mosaicism was detected. Here, the chromosome 15 long arm segment (15q13→qter) was involved in a terminal translocation with the long arm of chromosome 5 (table 1 and fig. 1). Again, telomeric sequences were detected at the breakpoint junction of the derived chromosome, as well as at both ends of the derived chromosome (fig. 2*c*). Although hybridization to the 15qter telomere was not observed in the cell shown, it was observed in other cells examined.

In case 3, extra material was present on the distal long arm of one copy of chromosome 2 (table 1 and fig. 1). The extra material originated from chromosome 17 (data not shown). As in cases 1 and 2, hybridization with the telomere consensus sequence demonstrated the presence of interstitial telomeric sequences at the breakpoint junction (fig. 2*d*).

Case 4 exhibited mosaicism, with multiple cell lines containing abnormalities involving chromosome 18. The predominant cell line contained a ring chromosome consisting of all or most of a chromosome 18 and an additional segment of chromatin that may have originated from chromosome 9 (table 1 and fig. 1). This was the only cell line evaluated by in situ hybridization. Hybridization revealed the presence of telomeric sequences within the ring chromosome. In figure 2*e*, a doublet of hybridization is apparent, as expected if there is one site of hybridization on each chromatid of the mitotic chromosome. In many of the metaphase spreads examined, there was a single site of hybridization, as if the two chromatids were stacked on top of one another (data not shown). The origin of the telomere sequences could not be established. Presumably, they originated from either 18p or 18q. In spite of the presence of telomeric sequences within the ring chromosome, it appeared to be stable in circular form.

In case 5, stalks and satellites were present on the long arm of chromosome 1 (table 1 and fig. 1). The 1qs is a familial rearrangement. Two sets of telomeric sequences were observed at the satellited end of the rearranged chromosome 1 (fig. 2*f*). Presumably, the interstitial telomeric sequences are those normally present at 1qter, and the terminal telomere was contributed by the acrocentric short arm (compare inset of fig. 2*f*). Two rounds of signal amplification were performed in this experiment, which accounts for the increased level of nonspecific background.

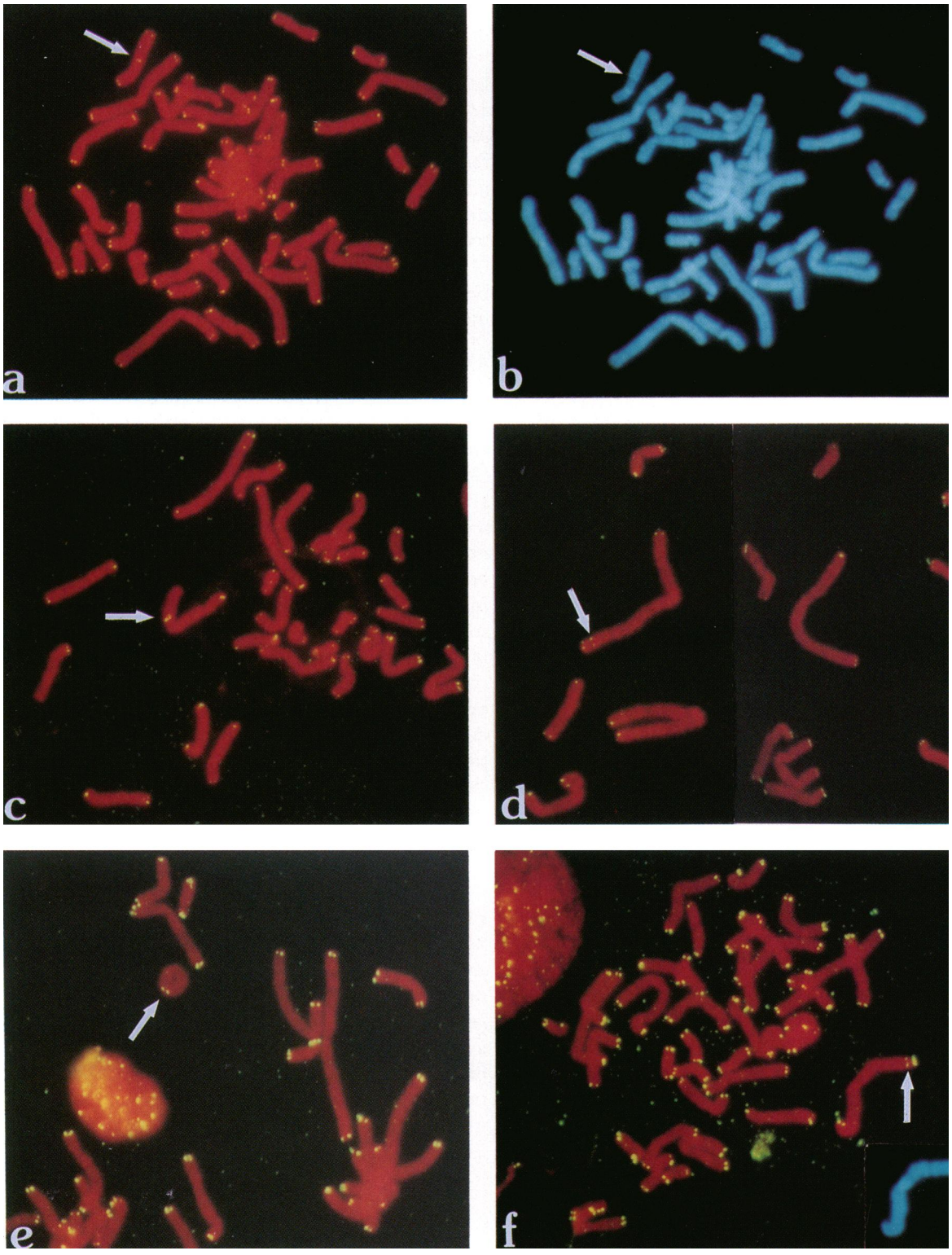
Absence of Interstitial Telomeres

Cases 6 and 7 involved derived chromosomes with extra material, of unidentified origin, attached to one end of an apparently intact recipient chromosome (2p+ and 20p+; table 1 and fig. 1). Both rearrangements originated de novo. Each derived chromosome contained only terminal telomeres; there was no evidence of interstitial telomeric sequences at the breakpoint junction of either abnormal chromosome (data not shown). Although both abnormal chromosomes are analogous to the rearrangements described in cases 1–3 in that all involve extra euchromatic material joined to a recipient chromosome with a terminal breakpoint, they differ in that interstitial telomeric sequences are absent. Thus, it appears that the chromosomes in cases 6 and 7 are products of reciprocal exchanges, maintaining the expected configuration of two terminal telomeres per chromosome. Alternatively, case 7 may represent a tandem duplication of chromosome 20 material.

Cases 8 and 9 were both balanced familial translocations with a terminal breakpoint (10q26.3 and 1p36.3, respectively; table 1 and fig. 1). In both cases, sites of hybridization were observed at the termini of the derived chromosomes, but no interstitial telomeric sequences were detected (data not shown). Thus, it appears that both translocations are reciprocal two-break rearrangements, although, in each case, one of the translocated segments is too small to be visualized in Giemsa-banded preparations.

Presence of Terminal Telomeres

In the cases where interstitial telomeric sequences were observed, the donor chromosomes involved in each rearrangement were absent (e.g., the centric segments of chromosome 15 in cases 1 and 2 and of chromosome 17 in case 3). Since each recipient chromo-


a
b
c
d
e
f

some exhibited three sets of telomeric sequences, the question arises as to whether a deleted chromosome lacking a terminal telomere can exist. This question was investigated by looking at three examples of terminal deletions. Cases 10, 11, and 12 exhibited deletions involving chromosomes 2q, 13q, and 14q, respectively (table 1 and fig. 1). The telomere probe was observed to hybridize to the terminus of each deleted chromosome (data not shown). Similar observations have been made by Lin et al. (1991). These results are consistent with expectations that, in order to be stable, a linear chromosome must have a functional telomere at each end.

Discussion

Nine rearrangements where additional material was joined to an apparently intact recipient chromosome (or chromosome end) were examined (cases 1–9). In cases 1–5, a novel chromosome structure was observed where interstitial telomeric sequences are present at the breakpoint junctions. The simplest explanation of the observed interstitial telomeric sequences is that the broken end of one chromosome is able to fuse to the microscopically intact terminus of a second chromosome. Such a structure has not been previously reported in humans, even after induced chromosome breakage (Cornforth et al. 1989). Because of the involvement of an acentric chromosome segment, this structure differs both from the telomeric associations observed in some tumor cells (Mitelman 1988, p. xviii; Schwartz et al. 1990) and from the telomeric fusions observed in evolutionary studies (Ijdo et al. 1991), both of which represent end-to-end fusions of intact chromosomes.

The existence of interstitial telomeric sequences has been suggested in several reports involving different classes of terminal rearrangements in human cells. In situ hybridizations of telomere probes were used to identify interstitial telomeric sequences, both in a telomeric association present in a giant-cell tumor of bone (Schwartz et al. 1990) and in a constitutional dicentric chromosome rearrangement involving an X chromosome and a chromosome 22 (Jedele et al. 1989). In addition, this type of chromosome structure was postulated in a jumping translocation involving chromosome 22 (Drake et al. 1985). Internal telomeric repeats also have been observed in vertebrate chromosomes. Chromosome fusion and fission within these repeated sequences appear to play an important role in evolution (Meyne et al. 1990; Ijdo et al. 1991).

The use of in situ hybridization extends the characterization beyond what is possible with conventional cytogenetic techniques, but it does not explain the molecular basis of the novel structure. Certainly, one or more of the chromosomes described may be a product of complex rearrangements involving multiple breaks. However, in light of the high frequency of the novel structure (five of nine rearrangements examined), it seems likely that such a structure may arise through a relatively simple mechanism. One possibility is that a break might occur in the recipient chromosome, removing some but not all of the telomeric consensus sequence. After removal of the cap structure, the broken end might participate in the formation of a phosphodiester linkage with another broken end. The remaining telomeric consensus sequences would yield a positive hybridization signal. Since the hybridizations reported here are not quantitative, this hypothesis cannot be addressed. In each case where interstitial telomeric sequences were identified, the centric segment of the second chromosome involved was lost. Therefore, it could not be examined for the presence or absence of a telomere at the deleted end. This hypothesis does, however, allow for the possibility that the rearrangements are products of reciprocal exchanges.

Another possibility is that the telomere cap structure might be disrupted without chromosome breakage, through the unfolding of a putative hairpin loop or the transient absence of telomere-binding proteins. Such a disruption might permit the chromosome end to be joined to a broken segment of another chromosome.

Figure 2 In situ hybridization of the telomere consensus sequence, demonstrating the presence of interstitial telomeric sequences. In each panel, the arrow indicates the breakpoint in the abnormal chromosome. The yellow fluorescein signal denotes telomeric sequences within propidium iodide–stained chromosomes. *a*, Case 1, der(19). *b*, Same metaphase spread shown in panel a, showing faint banding pattern produced by DAPI. *c*, Partial metaphase spread from case 2, showing the der(5). *d*, Partial metaphase spread from case 3, showing the der(2) (arrow) and the normal chromosome 2 to its right. *e*, Partial metaphase spread from case 4, showing the ring chromosome. *f*, Case 5, 1qs. The inset is included to illustrate the chromosome morphology (stalks and satellites) of the DAPI-stained 1qs chromosome.

A consequence of this explanation is that the rearrangements are products of nonreciprocal exchanges.

The novel chromosome structure described in this study is significant in the clinical interpretation of chromosome rearrangements possessing terminal breakpoints. In such rearrangements, the recipient chromosome may or may not contribute to the phenotype, depending on whether a partial monosomy is present. For example, in case 3, interstitial telomeric sequences exist, and, presumably, there is no deletion of functional gene sequences from 2qter. Alternatively, in case 6, no interstitial telomeric sequences were observed in the 2p+ chromosome. It follows that some sequences have been deleted from 2pter, to give a situation where both partial monosomy (2p) and partial trisomy (chromosome unknown) are present. Depending on the extent of the resulting partial monosomy of 2p, the deletion may contribute to the phenotype of the individual. In attempting to correlate various chromosome rearrangements with particular phenotypes, it is valuable to differentiate between a pure partial trisomy (or monosomy) and a situation that is complicated by the presence of both duplication and deletion. In situ hybridization provides a direct method to accomplish this. However, in addition to an assessment of the duplication or deletion of particular sequences, it is possible that position effects of unknown etiology may be operational in determining phenotype.

The chromosomal elements critical to chromosome structure and function are the centromere and the telomere. It appears that both structures may exist in a nonfunctional state. In dicentric chromosomes, it is common for one centromere to be inactivated, thus preserving mitotic stability of the structurally abnormal chromosome. Likewise, telomeric sequences may exist within chromosomes without performing the traditional functions of a telomere. The identification of the primary sequences that form a scaffold for centromeres and telomeres represents an important step in understanding chromosome structure. However, it is clear that chromosome function involves complex interactions that go beyond what can be understood from primary sequences.

Acknowledgments

We gratefully acknowledge the excellent technical work of Marie Haren, Margaret McCann, Kathleen Sweeney, and Bruce Tyler, and, in addition, we thank Mary Kochera for careful reading of the manuscript. We thank Drs. Arthur Zinn, Eileen Stork, and Irwin Schafer for their cooperation in obtaining patient specimens. We thank Imagenetics for providing painting probes and information.

References

Blackburn EH (1991) Structure and function of telomeres. Nature 350:569–573

Cornforth MN, Meyne J, Littlefield LG, Bailey SM, Moyzis RK (1989) Telomere staining of human chromosomes and the mechanism of radiation-induced dicentric formation. Radiat Res 120:205–212

Drake VJ, Tunnell S, Ward BE, Robinson A (1985) Unblanced, unstable translocations of 22q. Am J Hum Genet 37 [Suppl]: A91

Gray JT, Celander DW, Price CM, Cech TR (1991) Cloning and expression of genes for the Oxytricha telomere-binding protein: specific subunit interactions in the telomeric complex. Cell 67:807–814

Greider CW, Blackburn EH (1987) The telomere terminal transferase of Tetrahymena is a ribonucleoprotein enzyme with two kinds of primer specificity. Cell 51:887–898

——— (1989) A telomeric sequence in the RNA of Tetrahymena telomerase required for telomere repeat synthesis. Nature 337:331–337

Holmquist GP, Dancis B (1979) Telomere replication, kinetochore organizers, and satellite DNA evolution. Proc Natl Acad Sci USA 76:4566–4570

Ijdo JW, Baldini A, Ward DC, Reeders ST, Wells RA (1991) Origin of human chromosome 2: an ancestral telomere-telomere fusion. Proc Natl Acad Sci USA 88:9051–9055

Jedele KB, Michels VV, Thibodear SN, Dewald GW, Wagner KV, Schad CR, Stesin M, et al (1989) Demonstration of telomeric DNA at the fusion site in a constitutional dicentric X;22 chromosome translocation. Am J Hum Genet 45 [Suppl]: A102

Lin S-P, Gandelman K, Ijdo JW, Yang-Feng TL (1991) Molecular evidence of telomere sequences present at the broken ends of terminal rearranged chromosomes. Am J Hum Genet 49 [Suppl]: 298

Meyne J, Baker RJ, Hobart HH, Hsu TC, Ryder OA, Ward OG, Wiley JE, et al (1990) Distribution of non-telomeric sites of the $(TTAGGG)_n$ telomeric sequence in vertebrate chromosomes. Chromosoma 99:3–10

Meyne J, Ratliff RL, Moyzis RK (1989) Conservation of the human telomere sequence $(TTAGGG)_n$ among vertebrates. Proc Natl Acad Sci USA 86:7049–7053

Mitelman F (1988) Catalog of chromosome aberrations in cancer, 3d ed. Alan R Liss, New York

Morin GB (1989) The human telomere terminal transferase enzyme is a ribonucleoprotein that synthesizes TTAGGG repeats. Cell 59:521–529

Moyzis RK, Buckingham JM, Cram LS, Dani M, Deaven LL, Jones MD, Meyne J, et al (1988) A highly conserved

repetitive DNA sequence, $(TTAGGG)_n$, present at the telomeres of human chromosomes. Proc Natl Acad Sci USA 85:6622–6626

Muller HJ (1939) The remaking of chromosomes. Collecting Net Woods Hole 13:181–195

Park VP, Gustashaw KM, Wathen TM, Zinn AB, Rush PW (1991) Interstitial telomere sequences in a patient with Prader-Willi syndrome and a jumping translocation. Am J Hum Genet 49 [Suppl]: 305

Pinkel D, Landegent J, Collins C, Fuscoe J, Segraves R, Lucas J, Gray J (1988) Fluorescence in situ hybridization with human chromosome-specific libraries: detection of trisomy 21 and translocations of chromosome 4. Proc Natl Acad Sci USA 85:9138–9142

Rivera H, Zuffardi O, Gargantini L (1990) Nonreciprocal and jumping translocations of 15q1→qter in Prader-Willi syndrome. Am J Med Genet 37:311–317

Schwartz HS, Allen GA, Butler MG (1990) Telomeric associations. Appl Cytogenet 16:133–137

Zakian VA (1989) Structure and function of telomeres. Annu Rev Genet 23:579–604