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ARTICLE

FISHing for mechanisms of cytogenetically defined terminal deletions using chromosome-specific subtelomeric probes

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Cytogenetically defined terminal deletions are thought to be a major, yet underappreciated, cause of mental retardation and multiple congenital anomalies. The mechanisms by which terminal deletions arise and are stabilized are not completely understood; although all ends of human chromosomes must have a telomeric cap to be stable. At least three mechanisms exist to maintain chromosome ends with cytogenetically defined terminal deletions: stabilization of terminal deletions through a process of telomere regeneration (termed 'telomere healing'), retention of the original telomere producing interstitial deletions, and formation of derivative chromosomes by obtaining a different telomeric sequence through cytogenetic rearrangement (termed 'telomere capture'). We used chromosome-specific subtelomeric probes and FISH to characterize cytogenetically defined terminal deletions in patients with 1p36 monosomy. Based on the current resolution of these subtelomeric probes, our results indicate that cytogenetically defined terminal deletions of 1p36 are likely to occur through all three mechanisms, although we speculate that the majority of cases were stabilized through telomere regeneration. These results demonstrate the use of chromosome-specific subtelomeric probes as an efficient first step toward uncovering the mechanisms that result in the stabilization of cytogenetically defined terminal deletions.
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Introduction

A better understanding of the mechanisms which result in terminal deletions may be extremely valuable since the majority of all cytogenetically visible deletions involve the distal, telomeric bands of the chromosomes.[1] Any abnormality at the telomere can be particularly detrimental since human telomeres are the most gene-rich regions in the entire genome, making telomeric deletions potentially more clinically significant than similar sized deletions at other chromosomal locations.[2] Several examples of small telomeric deletions have been described.[3–8] Telomeric regions of the human genome are of particular interest in clinical cytogenetics since rearrangements of these regions are difficult to identify using conventional chromosome banding technology. Between 5% and 18% of patients with apparently normal karyotypes and unexplained mental retardation or dysmorphic features may actually have submicroscopic deletions at the telomere and/or cryptic telomere rearrangements.[6–8] Recently with the advent of molecular cytogenetic and DNA technologies, it has been possible to investigate the terminus in cytogenetically visible, terminal deletions.[9,10]

In the past decade, telomeres have been reported to be involved in several important functions.[11,12] Among these is the association of telomeres in meiosis suggesting that they play an essential role in homologue pairing and recombination, and possibly recombination between non-homologous chromosome ends.[11] The overall structure of human telomeric DNA recently has begun to be delineated. Every human chromosome is capped with between three and 20 kb of tandemly repeated $(TTAGGG)_n$ sequences.[13] Just proximal to this region, approximately 100–300 kb of additional telomere associated repeat (TAR) sequences are found.[14]

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Since the telomere is necessary for the stability of the chromosome, broken chromosomes (ie deletions) must retain or acquire this 'cap'. The first possibility is that 'terminal' deletions are really interstitial deletions that retain their original telomere.[10] Secondly, for those cases that are truly deleted for the telomere, two major mechanisms have been postulated to restore a lost telomere:

(1) *de novo* synthesis of telomeres by telomerase to 'heal' the chromosome,[15–17] and

(2) homologous recombination to 'capture' a telomere from another chromosome.[18,19]

Telomere healing has been demonstrated through sequence analysis of terminal deletions which showed that *de novo* telomeric repeats were synthesized directly to the unique sequences that remained.[15,17] Telomere capture[18] could potentially occur between sister chromatids, homologues, or nonhomologous chromosome ends. The results of telomere capture events are derivative chromosomes.[18,19] It has been postulated that sequence homology of the TAR regions between non-homologous chromosomes may predispose to these telomere capture events. Numerous examples exist in the literature of unbalanced, non-reciprocal, *de novo* derivative chromosomes as constitutional abnormalities. It is likely that these derivative chromosomes represent the outcome of telomere capture. It is unclear whether telomere healing, through *de novo* synthesis of the telomeric repeats, or telomere capture, through homologous recombination, is the predominant mechanism for stabilizing chromosome breaks in humans. TAR sequences can be polymorphic between homologues,[20] which may predispose to chromosome breakage that may stabilize through either telomere healing or telomere capture. Mispairing and recombination of chromosomes in meiosis, due to shared TAR sequences, may occasionally resolve as a translocation. The non-reciprocal nature of telomere capture events would lead to unbalanced derivative chromosomes, that, at the level of the light microscope, appear to be terminal deletions.

Recently, a complete set of human chromosome-specific subtelomeric probes was developed that provides ten times greater resolution at chromosome ends than standard cytogenetic banding.[21–23] These subtelomeric probes have been developed for nearly every human chromosome arm. A set of chromosome-specific subtelomeric probes is now commercially available as part of a diagnostic device that allows for the analysis of 41 subtelomeric regions on a single microscope slide.[8,22,23] These subtelomeric probes are all located within 400 kb of the telomere (Cytocell Ltd, Oxford, UK). The use of these subtelomeric probes in fluorescence *in situ* hybridization (FISH) provides the most rapid and sensitive means to date for initially characterizing cytogenetically defined terminal deletions.

In this study, we performed FISH for 41 subtelomeric regions on 33 patients with 1p36 monosomy using these novel diagnostic devices. The goal of the study was to initially characterize this group of cytogenetically defined terminal deletions in order to ultimately identify the mechanisms involved in stabilizing the deletions in patients with monosomy 1p36.

Materials and methods

Patients

Thirty-three individuals with 1p36 deletions, previously collected as part of a larger study of 1p36 monosomy, were investigated.[24] The patients consist of 23 females and 10 males coming from diverse ethnic backgrounds. Informed consent was obtained from the parents or guardians of all patients consistent with procedures approved by the Institutional Review Board of Baylor College of Medicine. Thirty of these patients (patients 1–30) have been previously reported.[24]

FISH

Metaphase chromosome preparations of peripheral blood lymphocytes or lymphoblastoid cells were prepared using standard cytogenetic laboratory procedures. FISH for 41 subtelomeric regions was performed on a single microscope slide for each patient using the Chromoprobe Multiprobe®-T System (Cytocell Ltd, Oxford, UK) according to the manufacturer's specifications and as previously published.[22]

Briefly, for each patient sample, fixed metaphase chromosome preparations were spotted on a microscope slide that had been divided into 24 individual squares. Each of the 24 squares represents a hybridization area for a single chromosome (chromosomes 1–22 plus an additional box for the sex chromosomes, and one square is empty). A multiprobe device, complementary to the subdivided slide with 24 raised square surfaces, was used to ensure hybridization of the p-arm and q-arm chromosome-specific probe sets only within the appropriate box. For example, hybridization with the chromosome 1p and 1q probes was only performed in box 1; hybridization with the 2p and 2q probes only in box 2, etc. For the acrocentric chromosomes, probes are available for only the long arms, since the acrocentric short arms share most of their repetitive DNA sequences and have no known unique sequences. For the sex chromosomes, a single set of probes hybridize to the pseudoautosomal regions of both the X and Y chromosomes. Hybridization was performed as specified by the manufacturer (Cytocell Ltd, Oxford, UK).

Multiprobe devices containing either directly or indirectly labeled probes were used. Signal amplification for indirectly labeled probes was performed using anti-digoxigenin antibodies conjugated to FITC for p arm-specific probes (fluoresces green) and anti-biotin antibodies conjugated to Cy3 for q arm-specific probes (fluoresces red). Cells were counterstained with DAPI and viewed with a Zeiss Axiophot fluorescent microscope equipped with single-band-pass filters as well as a triple-band-pass filter that allows one to

visualize single colors or multiple colors, respectively. Digital images were captured using a Power Macintosh G3 system and MacProbe version 4.0 (Perceptive Scientific Instruments Inc, League City, TX, USA). Images were printed using a color/monochrome Phasar II SDX printer (Tectronix, Wilsonvill, OR, USA). At least two complete and unambiguous metaphase spreads were analyzed in each box for detection of the presence or absence of hybridization with the p and q arm probes specific for that individual chromosome.

All cases involving derivative chromosomes were confirmed by a second FISH experiment using chromosome-specific subtelomeric probes for the individual chromosome involved in each derivative 1p. Upon confirmation of a patient's derivative 1p chromosome, available parental samples were screened by FISH to see if the resulting 1p36 deletion was due to malsegregation of a cryptic parental translocation and to eliminate the possibility of segregation of an apparently benign familial polymorphism.[25] For these experiments, probes spotted to a single coverslip (Chromoprobe®-T, Cytocell Ltd, Oxford, UK) were used according to the manufacturer's specifications. These cases were analyzed by scoring at least 30 complete and unambiguous metaphase spreads.

Cases that showed a terminal deletion of 1p with no other detectable cytogenetic rearrangement were screened by FISH for the presence of telomeric repeat sequences on the p arms of both chromosomes 1. For these FISH experiments, a $(TTAGGG)_n$ repeat probe (All Human Telomeres, Oncor, Gaithersburg, MD, USA) was used according to the manufacturer's specifications. At least two metaphase spreads from each of these patients were analyzed for hybridization with this probe. Chromosomes 1 were identified by the DAPI bright appearance of the long arm, pericentromeric heterochromatin.

Results

Based on prior molecular analysis, the deletion sizes were known in 30 of 33 patients studied;[24] however, the termini had not been characterized. These cases were further studied by FISH with chromosome-specific subtelomeric probes. This allowed for the classification of these cases into groups based on the structure at the end of the deleted chromosome 1 (Table 1). Prior to the subtelomeric FISH, six patients were known to have rearranged chromosomes and were included as controls: one case had a satellited 1p, one case was the unbalanced segregant from a t(1;22)pat,[26] one case was previously determined to have 1q sequences on distal 1p based on the G-banding pattern and confirmed by a chromosome 1 paint, and three patients had known interstitial deletions, based on molecular studies.[24]

The subtelomeric FISH identified five patients carrying *de novo* rearrangements indicative of telomere capture (Figure 1). These included three cases of derivative chromosomes with deletion of 1p and the addition of 1q sequences (Figure 1A), one case with 2p telomeric sequences on the derivative 1p (Figure 1B), and one case with Xp sequences replacing the deleted 1p telomeric sequences (Figure 1C). FISH on the parents' chromosomes using the same subtelomeric probes showed all five of these derivative chromosomes to be *de novo* (data not shown). The satellited 1p chromosome which carries 15p sequences was not detected by this assay, due to lack of chromosome-specific subtelomeric FISH probes for the acrocentric short arms, but was also shown to be *de novo* (data not shown). Additionally, 22q terminal sequences were not detected on the der(1)t(1;22)pat chromosome, and were not detected subsequently on the father's derivative chromosome 1 (data not shown), presumably due to rearrangement of 22q distal to the region identified by the subtelomeric probe used. Five of the 33 patients showed no deletion of the 1p chromosome-specific subtelomeric probe, indicating interstitial deletions (Figure 1D), and 21 patients showed deletions of the 1p subtelomeric probe with no other detectable telomeric rearrangement (Figure 1E). FISH experiments using a $(TTAGGG)_n$ telomeric repeat probe confirmed the presence of telomeric repeats at both ends of these 21 terminally deleted chromosomes 1 (Figure 1F). This last group of cases is by far the largest and provides preliminary evidence consistent with

Table 1 Results of subtelomeric FISH studies in 33 patients with monosomy 1p36

No. cases	*G-banding*	*Molecular characterization*[24]	*Telomere FISH*	*Interpretation*
21[a]	del(1)(p36.3)	del(1)(p36.3)	del(1)(p36.3)	terminal
3[b]	del(1)(p36.3)	del(1)(p36.3p36.3)	del(1)(p36.3p36.3)	interstitial
2[c]	del(1)(p36.3)	del(1)(p36.3)	del(1)(p36.3p36.3)	interstitial
1[d]	der(1)t(1p;1q)	del(1)(p36.3)	der(1)t(1p;1q)	derivative
2[e]	del(1)(p36.3)	del(1)(p36.3)	der(1)t(1p;1q)	derivative
1[f]	del(1)(p36.3)	del(1)(p36.3)	der(1)t(Xp;1p)	derivative
1[g]	del(1)(p36.3)	del(1)(p36.3)	der(1)t(1p;2p)	derivative
1[h]	der(1)t(1p;15p)	del(1)(p36.3)	del(1)(p36.3)	derivative
1[i]	der(1)t(1p;22q)pat	del(1)(p36.3)	del(1)(p36.3)	derivative

[a]Cases 2, 4, 6–9, 11, 12, 14, 16, 20–25, 27, 28, and 30, previously reported,[24] cases 32 and 33, this study; [b]Cases 1, 3 and 18, previously reported;[24] [c]Cases 10 and 17, previously reported;[24] [d]Case 15, previously reported;[24] [e]Cases 5 and 29, previously reported;[24] [f]Case 31, this study; [g]Case 26, previously reported;[24] [h]Case 19, previously reported;[24] [i]Case 13, previously reported.[24,26]

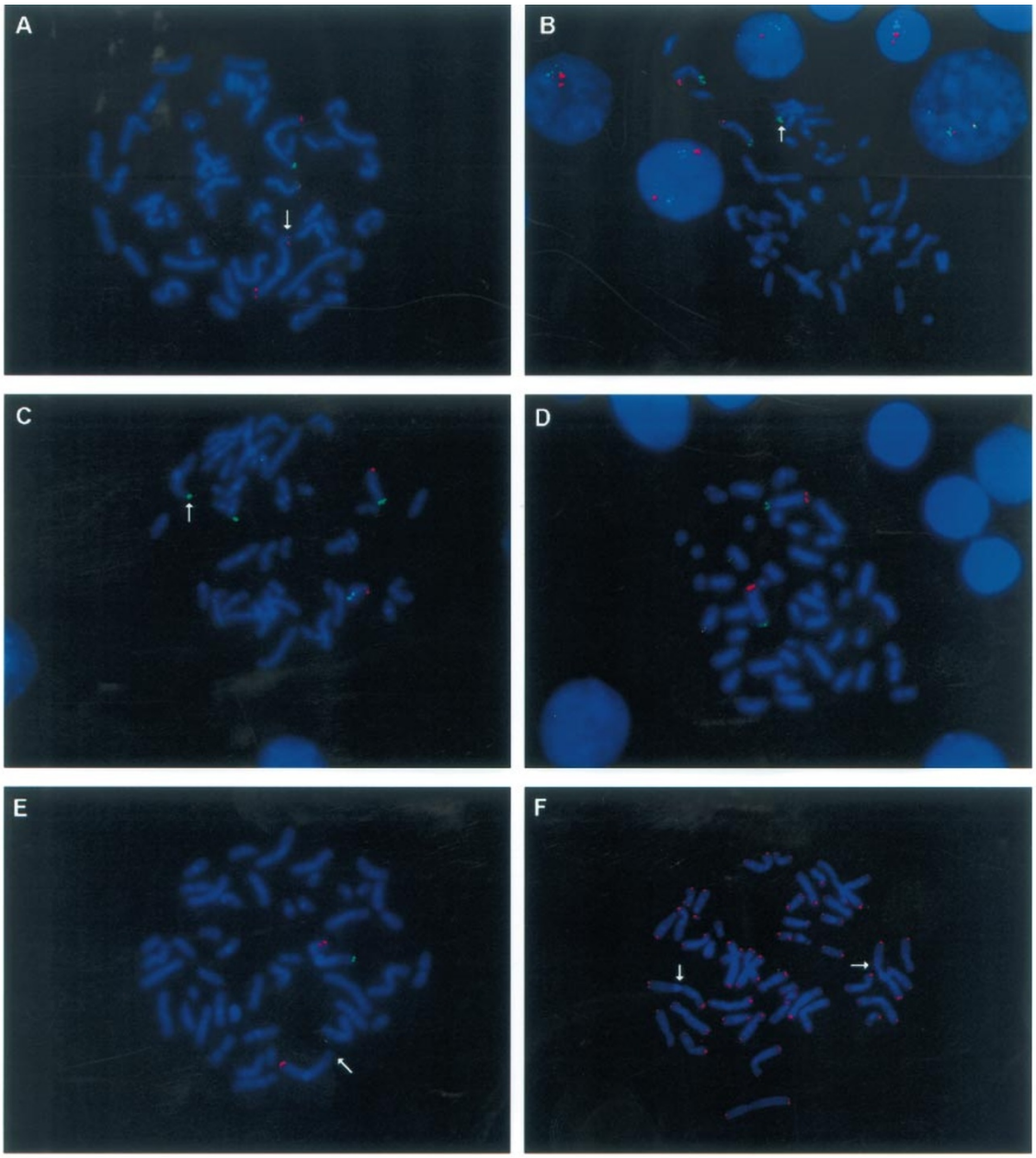

Figure 1 Representative FISH examples for each potential mechanism. Probes specific for p arms have a green signal and q arm-specific probes show a red signal. (**A**) Metaphase imaged from box 1 of the slide for Case 15 which represents one of three cases in which 1q sequences (probe 160H23) were present on the derivative chromosome 1p (arrow). (**B**) Metaphase imaged from box 2 of the slide for Case 26 showing normal hybridization of the chromosome 2p and 2q subtelomeric probes to the two normal chromosomes 2 with an additional green signal on chromosome 1 (arrow). This indicates 1p sequences were replaced by 2p sequences (probe dJ892G20) since 1p telomeric sequences were deleted from one chromosome 1 in box 1 of the same slide (data not shown). (**C**) Metaphase imaged from the XY box of the slide for Case 31 showing normal hybridization to both X chromosomes and an additional Xp signal on chromosome 1 (arrow). This indicates 1p sequences were replaced by Xp sequences (probe 98C4) since 1p telomeric sequences were deleted from one chromosome 1 in box 1 of the same slide (data not shown). (**D**) Metaphase imaged from box 1 of the slide for Case 18 which represents one of five cases identified as having an interstitial deletion due to the presence of 1p and 1q signals on both chromosomes 1. (**E**) One of 21 cases (Case 33 shown) revealing a deletion for this very distal 1p subtelomeric probe (CEB108) (arrow) with no other cytogenetic rearrangement detected by this assay. (**F**) Metaphase from one of the 21 cases (Case 33 shown) that was deleted for the 1p subtelomeric probe but showed hybridization with a $(TTAGGG)_n$ telomeric repeat probe at the ends of both chromosomes 1. The arrows indicate the locations of both chromosomes 1 identified by characteristic morphology and pericentromeric DAPI staining of the 1q heterochromatin.

chromosome healing of cytogenetically defined terminal deletions of the short arm of chromosome 1.

Discussion

Recently, Verma and Macera addressed whether or not cytogenetically defined terminal deletions are truly terminal or interstitial (original telomere retained).[27] In current practice of clinical cytogenetics, breakpoints in terminal bands are written as terminal, so as to conform to standard cytogenetics nomenclature indicating loss of all segments distal to the breakpoints, but it is assumed that the rearranged chromosome has a telomere at its terminus, conferring stability.[28] Original reports of terminal deletions were made prior to the use of chromosome banding techniques[29,30] and thus were large and easily distinguished from the normal homologue based on overall size of the chromosome. With the advent of banding techniques and high resolution chromosome analysis, many more subtle deletions of terminal bands were recognized and allowed the delineation of microdeletion syndromes.[31,32] One, newly recognized, deletion syndrome is monosomy 1p36.[24,26,33] This deletion syndrome is quite common in the population, with an estimated incidence of 1 in 10 000 newborns.[26] Herein, we investigated 33 patients with cytogenetically defined terminal deletions of 1p36 and found interstitial deletions, and evidence for telomere healing and telomere capture mechanisms.

Six cases were discovered to be *de novo* derivative chromosomes, likely to represent telomere capture. For these, there may be homology between the 1p telomere and a specific subset of other chromosome ends that allowed for recombination events to occur leading to the derivative chromosomes seen. Therefore, in an attempt to identify the chromosomes that are most commonly involved in rescuing cytogenetically defined terminal deletions of 1p by telomere capture, we compared our findings with other reported cases involving 1p deletions and/or translocations. Table 2 contains a list of all reported derivative chromosomes involving 1p36 that resulted in deletion.[24,26,34–44] Translocation events involving 1p have been reported to occur with a wide variety of chromosome ends. Some cytogenetic rearrangements have been reported more than others and a better understanding of these particular rearrangements could provide insights into the formation and stabilization of cytogenetically defined terminal deletions of chromosome 1p through telomere capture. However, caution should be exercised since the published literature may have an ascertainment bias because it may reflect only those cytogenetic rearrangements associated with survival or those identified as an obvious chromosomal abnormality using standard cytogenetic banding techniques. The increasing use of the much more sensitive FISH technology should allow for a more accurate representation of all of the chromosomes that are involved in translocation events with 1p36 and thus potentially involved in telomere capture.[40]

Table 2 Chromosomes involved in derivatives resulting in 1p36 deletions

Chromosome	*No. of cases*	*No. of cases involving p am*	*No. of cases involving q am*	*Reference*
1	4		4	34, this study
2	1	1		this study
3				
4				
5				
6				
7				
8				
9	1	1		36
10				
11				
12				
13	2	1	1	37, 38
14				
15	4	3	1	35, 39, 40, this study
16	1	1		41
17				
18				
19	1		1	38
20	1	1		42
21	1	1		43
22	1		1	24, 26
X	1	1		this study
Y	1	1		44

For those 21 patients who showed a terminal deletion through the use of the 1p subtelomeric FISH probe, some may actually have interstitial deletions that might be identified if a more distal unique 1p probe were used than the one utilized here. However, it may be difficult to identify a more distal 1p sequence that is suitable for use as a unique chromosome-specific subtelomeric probe due to the close proximity of this 1p probe to the TAR sequences that are potentially shared among chromosomes. Likewise, it is still possible that translocations of shared TAR sequences, distal to the unique sequence probes used in this study, occurred but were not detected in these cases.[45] Our inability to detect the t(1;22)pat with this FISH assay may reflect such a case. As these probes become available, hybridization of 1p TAR sequences and other chromosomal TAR sequences to the chromosomes of these patients may reveal that telomere capture has occurred. Conversely, based on the very distal nature of the subtelomeric probe used in this study and previous reports of the healing of chromosome 16p breaks,[15–17] it is likely that the major mechanism by which cytogenetically defined terminal deletions of 1p are stabilized is by the *de novo* addition of terminal $(TTAGGG)_n$ repeats. Cases of this type have been confirmed by cloning the breakpoints and sequencing directly into the telomeric repeats.[15–17] This approach has also aided in the identification of sequences that are needed by telomerase to elongate a telomere[15–17] and may help identify regions of homology needed for recombination to produce 1p36 deletions. All

21 cases in our study were shown to have the telomeric repeat on distal 1p.

For the five patients with interstitial deletions, two were previously not known to be interstitial but were uncovered with the use of this very distal 1p probe. Cloning the breakpoints of these interstitial deletions may elucidate the mechanism of formation and uncover the possibility that low-copy repeat sequence homology was necessary or involved in producing the deletion.[46–50]

Although future studies that include cloning the breakpoints of these terminally deleted chromosomes should more precisely determine their structure, our preliminary findings demonstrate that potentially all three mechanisms stabilized these chromosome ends. This indicates that multiple mechanistic routes were taken to generate these deletions. Finally, 'FISHing' with chromosome-specific subtelomeric probes may be the best way initially to characterize other cytogenetically defined terminal deletions.

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BCB and CDK contributed equally to this work.

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