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Evidence for Structural Heterogeneity from Molecular Cytogenetic Analysis of Dicentric Robertsonian Translocations

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Summary

Most Robertsonian translocations are dicentric, suggesting that the location of chromosomal breaks leading to their formation occur in the acrocentric short arm. Previous cytogenetic and molecular cytogenetic studies have shown that few Robertsonian translocations retain ribosomal genes or β-satellite DNA. Breakpoints in satellite III DNA, specifically between two chromosome 14–specific subfamilies, pTRS-47 and pTRS-63, have been indicated for most of the dicentric 14q21q and 13q14q translocations that have been studied. We have analyzed the structure of 36 dicentric translocations, using several repetitive DNA probes that localize to the acrocentric short arm. The majority of the translocations retained satellite III DNA, while others proved variable in structure. Of 10 14q21q translocations analyzed, satellite III DNA was undetected in 1; 6 retained one satellite III DNA subfamily, pTRS-47; and 3 appeared to contain two 14-specific satellite III DNA subfamilies, pTRS-47 and pTRS-63. In 10/11 translocations involving chromosome 15, the presence of satellite III DNA was observed. Our results show that various regions of the acrocentric short arm, and, particularly, satellite III DNA sequences, are involved in the formation of Robertsonian translocations.

Introduction

Robertsonian translocations are the most common structural chromosome abnormality in humans (Hamerton 1975). At least 90% of Robertsonian translocations occur between nonhomologous acrocentric chromosomes and are dicentric, suggesting that they more likely result from short-arm recombination rather than centric fusions as initially proposed by Robertson (1916) (Niebuhr 1972; Daniel and Lam-Po-Tang 1976; Mattei et al. 1979; Cheung et al. 1990; Gravholt et al. 1992; Wolff and Schwartz 1992). The mechanism of Robertsonian translocation formation via recombination within acrocentric short-arm DNA has been suggested by several investigations (Ferguson-Smith 1967; Therman 1980; Guichaoua et al. 1986; Choo et al. 1988). The high frequency of t(13q14q) and t(14q21q) (≥80% of all Robertsonian translocations) has been hypothesized to reflect the presence of a homologous repetitive sequence on chromosomes 13 and 21, which is inverted in chromosome 14 and perhaps lends to preferential pairing and exchange among chromosomes 13, 14, and 21 (Choo et al. 1988; Therman et al. 1989). A recombination event between these homologous DNA sequences could possibly form a dicentric Robertsonian translocation.

Several satellite DNAs within the acrocentric centromere/short arm are shared by chromosomes 13, 14, and 21 (Choo et al. 1988, 1990*a*; Trowell et al. 1993). However, some satellite DNA subfamilies have been shown to localize in a chromosome-specific manner (Higgins et al. 1985; Choo et al. 1990*b*). At least two chromosome-specific satellite III DNA sequences are located on chromosome 14: pTRS-47, a proximal subfamily (Choo et al. 1990*a*), and pTRS-63, a distal subfamily (Choo et al. 1992). The short arm of chromosome 15 contains a specific satellite III DNA subfamily, as well (Higgins et al. 1985). In addition, a satellite I DNA subfamily has been mapped primarily to 13p11, but also in reduced amounts to chromosome 21, and possibly the centromeric regions of other acrocentric and nonacrocentric chromosomes (Kalitsis et al. 1993). Thus far, only satellite III DNA has been directly implicated in translocation formation in the two most common Robertsonian translocations (13q14q and 14q21q).

Several studies employing FISH of DNA probes specific for the acrocentric short arm have shown that most Robertsonian translocations retain a portion of band p11. The short-arm breaks occur proximal to the β-satellite DNA of p11 and distal to α-satellite (Cheung et al. 1990; Wolff and Schwartz 1992), presumably within the region containing satellite III DNA. Of 38 13q14q translocations previously studied, all generally

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retained satellite III DNA (Gravholt et al. 1992; Han et al. 1994), with breakpoints in 17 specifically located between pTRS-47 (proximal) and pTRS-63 (distal) (Han et al. 1994). In other studies, 20 14q21q translocations were both hybridization negative for ribosomal and β-satellite DNA (Cheung et al. 1990; Earle et al. 1992; Gravholt et al. 1992; Wolff and Schwartz 1992), and satellite III DNA was present in 14 translocations (Earle et al. 1992; Gravholt et al. 1992). Twelve translocations studied with two chromosome 14–specific satellite III DNA probes showed that in all of the translocations pTRS-47 was retained, but not pTRS-63 (Earle et al. 1992).

We have previously shown that centromeric activity apparently occurs nonrandomly in dicentric Robertsonian translocations (Sullivan et al. 1994). Although the features, if any, that may provide a functional advantage to a specific centromere are unknown, it is possible that translocation structure might correlate with the nonrandom centromeric activity observed in the dicentric translocations. In the present study, 36 different Robertsonian translocations were analyzed by FISH using DNA probes specific for the pericentromeric region of acrocentric chromosomes. The results indicate that the structure of the translocations can be heterogeneous not only among the different translocations but also within translocations involving the same acrocentric chromosomes. Our data do not indicate an obvious correlation between translocation structure and centromeric activity.

Material and Methods

Acquisition of Sample Material/Preparation of Metaphase Chromosomes

Most of the translocations included for study were ascertained either by prenatal diagnosis or spontaneous abortion. The origin and sample type for each translocation is included in tables 1–3. Sample material consisted of human lymphoblasts, fibroblasts, amniocytes, chorionic villi, and fresh lymphocytes. The majority of Robertsonian translocations were ascertained through the Center for Human Genetics, University Hospitals of Cleveland. Several cell lines were purchased from the National Institute of General Medical Sciences Human Genetic Mutant Cell Repository (Camden, NJ). The translocations initially studied by Wolff and Schwartz (1992) are denoted by an asterisk in tables 1–3. To delineate breakpoint regions in these cases, hybridizations were performed with probes—primarily D15Z1, pTRS-47, and pTRS-63—that had not been available in the lab during the previous study.

Metaphase chromosomes were obtained using standard methods (Moorhead et al. 1960; Anderson and Gusella 1984). Each translocation was identified as dicentric by using GTG-banding (Seabright 1971). The number of centromeres was confirmed, and an initial assessment of centromeric activity was made using dual-color FISH with α-satellite probes (Sullivan et al. 1994)

In Situ Hybridization

Four DNA probes for the acrocentric short arm were used for FISH analysis. Biotinylated probes D15Z1, specific for satellite III (classical satellite) DNA of chromosomes 15p11 and pB4, specific for β-satellite DNA on all of the acrocentric chromosomes (p11 and p13) were obtained from Oncor, Inc. DNA probes for two satellite III DNA subfamilies, pTRS-47 (localized to 14p11 and 22p11) and pTRS-63 (localized to 14p11), were provided by Dr. Andy Choo (Murdoch Institute, Melbourne, Australia) (Choo et al. 1990*a*, 1992). Dual-color FISH with α-satellite DNA probes was used to confirm the presence of two centromeric regions and to identify the active centromere for every case (Sullivan et al. 1994). Hybridizations with biotinylated and/or digoxygenin-labeled α-satellite DNA probes, D15Z1 (15 classical satellite) and the β-satellite DNA probe were performed according to manufacturer's protocols. In some cases, hybridizations with the short-arm probes were limited by sample availability.

Probe Labeling and Hybridization Conditions

pTRS-47 and pTRS-63 plasmid DNA was amplified by large-scale plasmid preparation and purified using the Qiagen Plasmid Maxi Kit (Qiagen). Amplified probe DNA was biotinylated by nick-translation (BioNick Labeling Kit; GibcoBRL) following manufacturer's instructions.

FISH with pTRS-47 and pTRS-63 was a modification of the method of Gravholt et al. (1992). In the translocations that did not include chromosome 14, pTRS-47 was used at lower stringency to detect the presence of satellite III DNA sequences within the other acrocentric short arms, but it was used at higher stringency in the translocations involving chromosomes 14 and/or 22. pTRS-63, a chromosome 14–specific satellite III DNA probe, was hybridized only to the translocations involving chromosome 14. The hybridization mixture consisted of 25 ng/μl DNA diluted in 50% formamide, 4× SSC, 10% dextran sulfate, which was spread on to slides, covered with a coverglass, and sealed with rubber cement. Target and probe DNAs were denatured simultaneously on a 70°C hot plate for 8–9 min, chilled on ice for 2 min (optional), and hybridized in a humid chamber for 24–48 h at 37°C. Slides were washed for 10–15 min in 0.4 × SSC/0.1% Tween-20 at 45°C–50°C (low stringency) or 60°C (high stringency), followed by a 5-min wash in 0.1 × SSC/0.1% Tween-20 at 45°C–50°C (low) or 55°C–60°C (high). DNA probes were detected with fluorescein-conjugated avidin (Oncor) for 15–30 min at 37°C, then washed for 5 min in 0.4 × SSC/0.1% Tween-20 or 1 × PBD (Oncor) at room temperature. If necessary, signals were amplified using biotinylated anti-avidin

Table 1

In Situ Results of t(13q14q) Studied for Presence of β-Satellite and Satellite III DNA

Case	Origin	Sample Type	β-Satellite DNA	pTRS-47	pTRS-63	Active Centromere, by FISH[a]
1	FAM	PB	−	+	−	14 (case 10)
2	FAM	A	nt	+	−	14 (case 11)
3	DN	A	−	+	−	13
4	UN	A	nt	+	−	14 (case 12)
5	UN	A	nt	nt	−	14
6	FAM	PB	−	+	−	13 (case 14)
7	DN	A	−	+	−	14 (case 15)
8	UN	A	−	+	−	14
9	DN	A	nt	+	−	14
10	UN	A	−	+	−	14 (case 16)
11	FAM	P	nt	+	−	14

NOTE.—nt = not tested; UN = unknown origin; DN = de novo; FAM = familial; A = amniocytes; P = placental tissue; and PB = peripheral blood lymphocytes. A plus sign (+) denotes positive hybridization with DNA probe; a minus sign (−) denotes negative hybridization with DNA probe.

[a] Numbers in parentheses refer to designated case number in study by Sullivan et al. (1994).

(Oncor) and fluoresceinated avidin. The DNA was counterstained with DAPI and propidium iodide and visualized with a Zeiss epifluorescence microscope. The DAPI counterstain allowed unequivocal identification of the translocation in every metaphase studied. Selected images were collected with a BioRad MRC 600 confocal scanning laser microscope or a Zeiss Axioplan microscope equipped with a cooled CCD camera and controlled by an Apple Macintosh computer.

Results

No translocation contained β-satellite DNA (fig. 1). Therefore, it can be assumed that the breakpoints in most Robertsonian translocations occur proximal to this repetitive DNA region. Three translocations showed no hybridization with the satellite III probes: one 14q21q translocation, one 13q22q translocation, and one 21q22q translocation, while the remaining translocations contained satellite III DNA between the two centromeres.

13q14q Translocations Are Structurally Similar

Ten translocations that were studied with pTRS-47 at high stringency retained this satellite III subfamily (fig. 2*a* and table 1). The intensity and amount of hybridization of pTRS-47 varied among translocations; however, in most cases, parental chromosomes were unavail-

Table 2

In Situ Results of t(14q21q) Studied for Presence of β-Satellite and Satellite III DNA

Case	Origin	Sample Type	β-Satellite DNA	pTRS-47	pTRS-63	Active Centromere, by FISH[a]
12	FAM	PB	−	+	−	14 (case 2)
13	FAM	PB/L	−	−	−	14 (case 3)
14	FAM	PB	nt	+	+	Random (case 4)
15	FAM	PB	nt	+	+	14 (case 5)
16[b]	FAM	PB	−	+	−	21 (case 7)
17	FAM	PB	−	+	+	14 (case 8)
18	FAM	F/L	−	+	−	14 (case 10)
19	FAM	P/PB/L	−	+	−	14 (case 11)
20	FAM	F/L	−	+	−	14 (case 13)
21	FAM	A	−	+	−	14 (case 12)

NOTE.—nt = not tested; FAM = familial; A = amniocytes; F = fibroblasts; L = lymphoblasts; P = placental tissue; and PB = peripheral blood lymphocytes. A plus sign (+) denotes positive hybridization with DNA probe; a minus sign (−) denotes negative hybridization with DNA probe.

[a] Numbers in parentheses refer to designated case number in study by Sullivan et al. (1994).

[b] Initially studied by Wolff and Schwartz (1992).

Table 3

In Situ Results of Remaining Translocations Studied for β-Satellite and Satellite III DNA

Case	Translocation	Origin	Sample Type	β-Satellite	D15Z1	pTRS-47	pTRS-63	Active Centromere, by FISH[a]
22	13q22q	UN	A	−	na	+	na	22[c]
23	13q22q	UN	A	nt	na	−	na	22[c]
24	21q22q	UN	PB	−	na	+	na	21[c]
25[b]	21q22q	FAM	L/F	−	na	−	na	21[c]
26	13q15q	FAM	PB	−	+	+	na	13 (case 2)
27	13q15q	UN	PB	−	+	+	na	13/15/both (case 3)
28	13q15q	UN	F	nt	+	+	na	13 (case 4)
29	13q15q	FAM	P/PB/L	−	+	+	na	13 (case 5)
30	14q15q	UN	L	−	+	+	+	14 (case 7)
31	14q15q	FAM	A	−	+	+	−	14
32	14q15q	FAM	P	−	+	+	−	Random (case 6)
33	14q15q	FAM	PB/L	−	−	+	−	15
34	15q21q	FAM	PB	−	+	+	na	21 (case 11)
35	15q21q	FAM	A	−	+	+	na	15
36	15q22q	FAM	PB/L	−	+	+	na	22 (case 12)

NOTE.—na = not applicable; nt = not tested; UN = unknown origin; FAM = familial; A = amniocytes; F = fibroblasts; L = lymphoblasts; P = placental tissue; and PB = peripheral blood lymphocytes. If the translocation did not involve chromosome 14, pTRS-63 was not used as a probe. A plus sign (+) denotes positive hybridization with DNA probe; a minus sign (−) denotes negative hybridization with DNA probe.

[a] Numbers in parentheses refer to designated case number in study by Sullivan et al. (1994).

[b] Initially studied by Wolff and Schwartz (1992).

[c] Studied by Sullivan et al. (1994).

able to quantitate this polymorphic repetitive subfamily in relation to the translocation chromosomes. No hybridization with pTRS-63 was detected in all 10 13q14q translocations (fig. 2*b*).

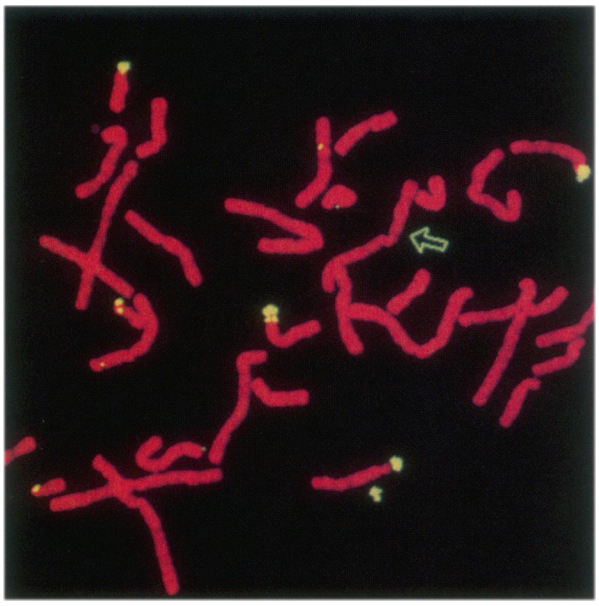

Figure 1 FISH, using β-satellite DNA probe. No Robertsonian translocation studied retained β-satellite DNA. A 14q15q translocation showed no hybridization with the β-satellite probe. Arrows denote translocation chromosomes.

14q21q Translocations are Structurally Heterogeneous

Satellite III DNA was undetected by FISH in only one 14q21q translocation (table 2, case 13; fig. 3, class I). Six translocations (cases 12, 16, 18–21) contained pTRS-47 only (fig. 3; fig. 4, class II), and both pTRS-47 and pTRS-63 hybridized to three translocations (cases 14, 15, 17; fig. 3; fig. 5, class III).

Breakpoint Heterogeneity in t(13q22q) and t(21q22q)

Two 13q22q and two 21q22q translocations were also analyzed with short-arm satellite probes. One 13q22q and one 21q22q translocation demonstrated hybridization with pTRS-47 at low stringency (table 3, cases 22 and 24). No detectable satellite III DNA was observed on one 13q22q (case 23) and one 21q22q (case 25) translocation. A marker chromosome contained within the cells of case 25 and previously shown to represent the reciprocal product of the exchange between chromosomes 21 and 22 (Wolff and Schwartz 1992) contained chromosome 22 α-satellite DNA, flanked distally on each arm of the marker by satellite III and β-satellite DNA (data not shown).

Translocations Involving Chromosome 15 Vary in Structure

Table 3 also includes the results of the 11 translocations involving chromosome 15 studied with FISH. Probe D15Z1 (chromosome 15–specific satellite III DNA) was used in all cases, while pTRS-47 was used at low-stringency hybridization conditions to detect sat-

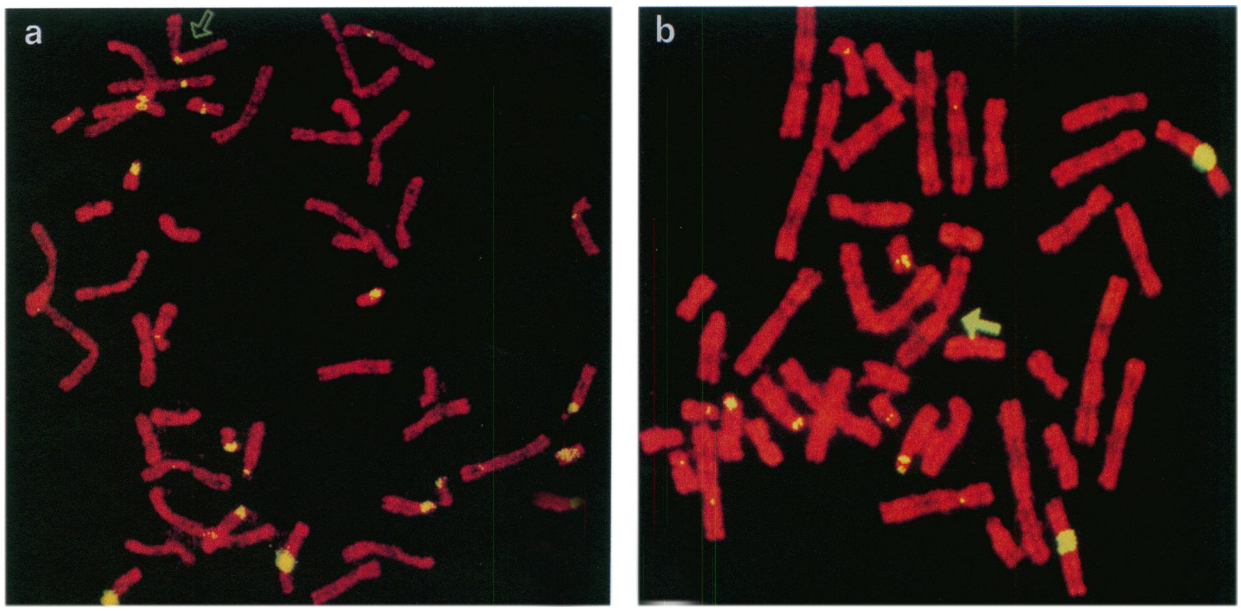

Figure 2 FISH, using satellite III DNA probes. All 13q14q translocations retained pTRS-47 (*a*), but not pTRS-63 (*b*). At low stringency, pTRS-47 hybridized to all acrocentric chromosomes and the heterochromatic region of chromosome 9. At moderate stringency, pTRS-63 often hybridized to chromosomes 9, 14, 15, and 22. Arrows denote translocation chromosomes.

ellite III DNA from the other acrocentric chromosome of the translocation. Satellite III DNA detected by pTRS-47 used at low stringency is very likely the chromosome 15–specific satellite III DNA detected by probe D15Z1. pTRS-63 (14-specific satellite III DNA) was used only in translocations involving chromosome 14.

Ten of 11 translocations involving chromosome 15 retained satellite III DNA from this chromosome (table 3; fig. 6*a*). Three of the four 13q15q translocations studied appeared to be structurally similar; they hybridized with both pTRS-47 and D15Z1. The centromeres of only one 13q15q translocation (case 26) were signifi-

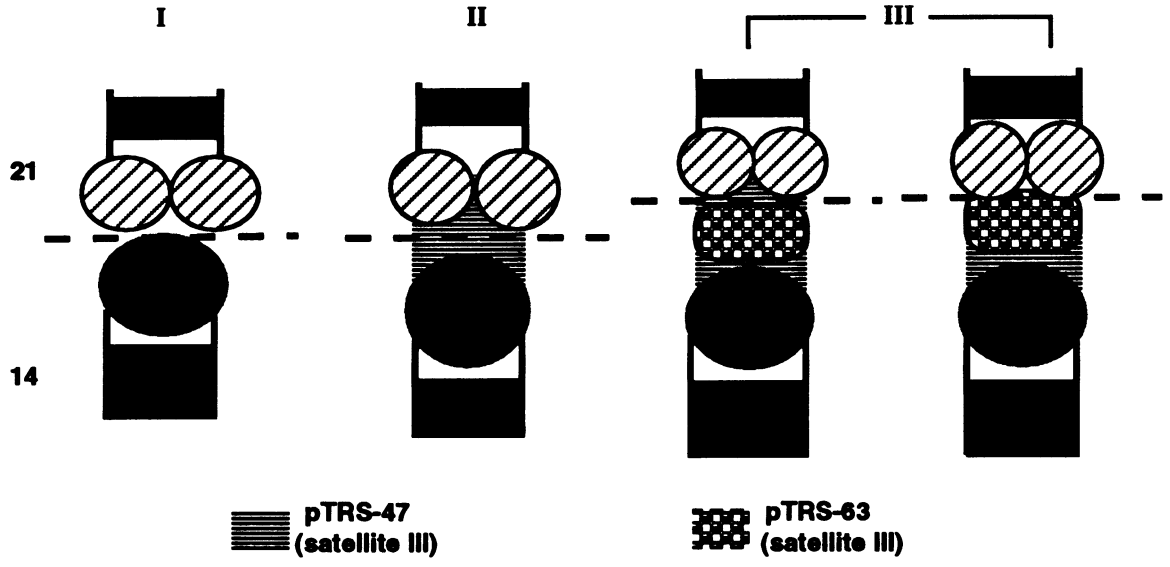

Figure 3 Structural classifications of 14q21q translocations. In this study, only one translocation had class I structure in which no satellite III DNA was detectable by FISH. The majority of 14q21q translocations belonged to class II in which pTRS-47 was retained, but not pTRS-63. In class III translocations, it was difficult to determine by FISH if satellite III DNA from only one of both acrocentric chromosomes was present, therefore, two possible structures may exist. Since the majority of Robertsonian translocations are stable dicentrics, an active centromere is denoted by a dark circle at the primary constriction, while an inactive centromere is represented by two, separated hashed circles.

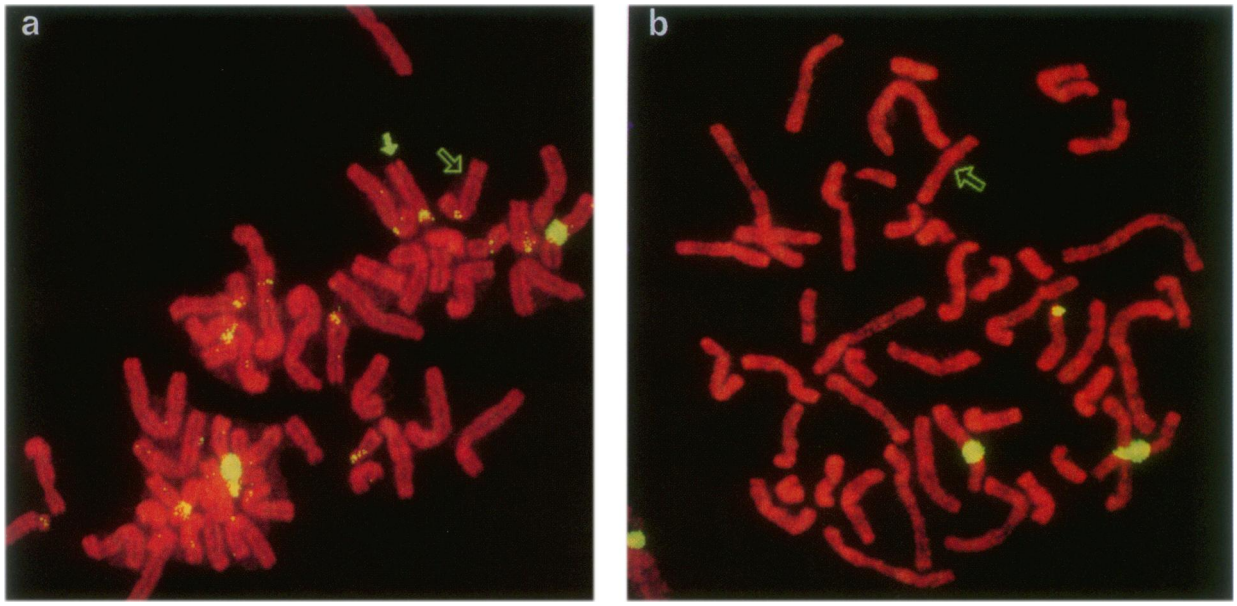

Figure 4 FISH analysis of class II 14q21q translocations. In six cases, pTRS-47 (*a*) was present, but pTRS-63 (*b*) was absent. The open arrow denotes the translocation, while the solid arrow points to the free chromosome 14.

cantly far apart to allow identification of hybridization signal from pTRS-47, which was not only closer to the chromosome 15 centromere than to the chromosome 13 centromere but in a similar location as the hybridization signal from D15Z1. Furthermore, the chromosome 13 α-satellite signal was noticeably smaller than both its normal homologue and the chromosome 15 α-satellite signal, suggesting that perhaps the chromosome 13 breakpoint lay within α-satellite DNA. Parental chromosomes were unavailable to compare size of the chromosome 13 centromeric signal. Satellite III DNA from chromosomes 14 and 15 were both retained in three of four 14q15q translocations that were studied. Only one of these three translocations (case 30) contained both

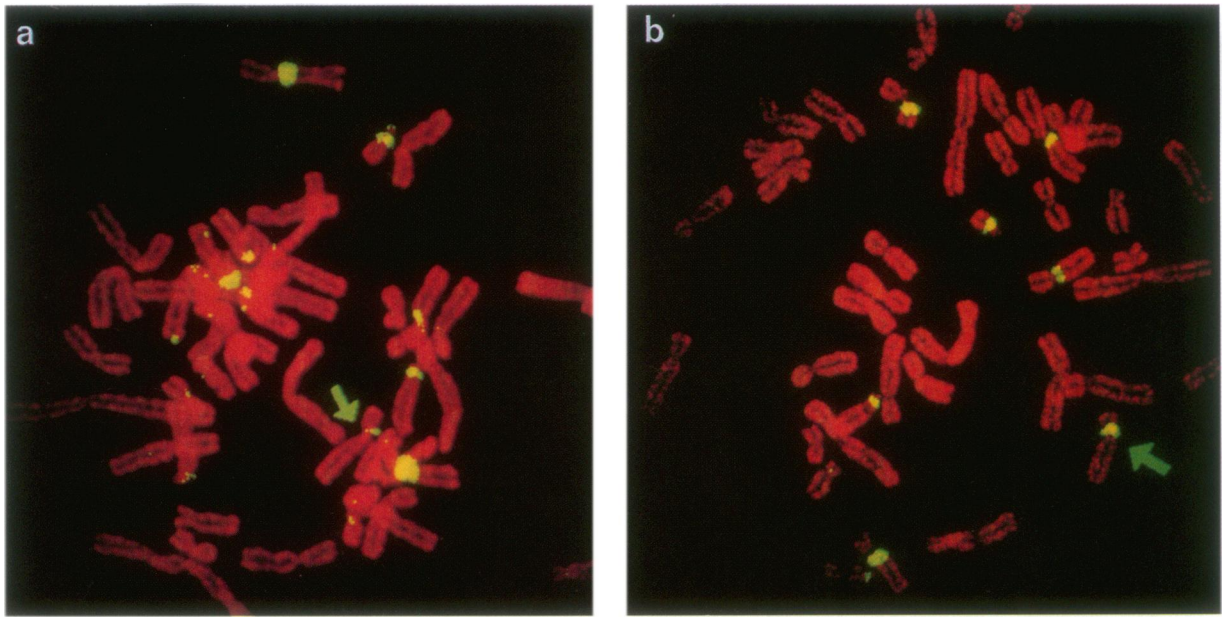

Figure 5 FISH analysis of class III 14q21q translocations. In three cases, hybridization signal from both pTRS-47 (*a*) and pTRS-63 (*b*) was present on the translocation. Arrows denote translocation chromosomes.

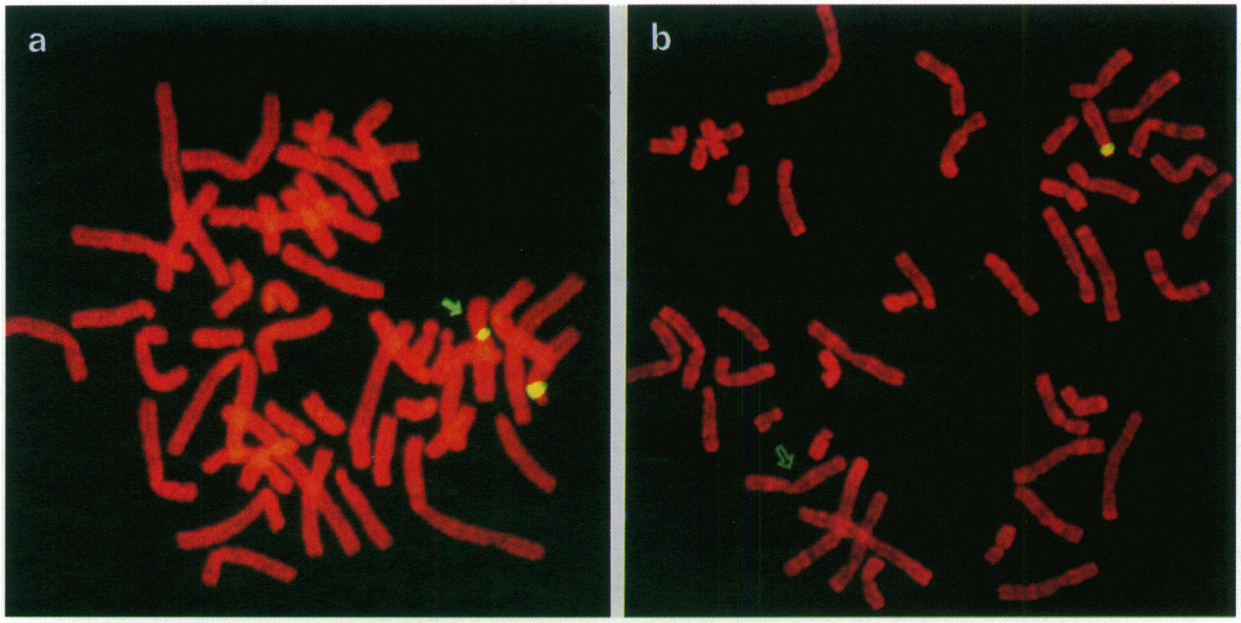

Figure 6 FISH analysis of translocations involving chromosome 15. Classical satellite (satellite III) DNA from chromosome 15 was present on most of the translocations (*a*); in case 33 (table 3), 15-specific satellite III DNA was absent (*b*). Arrows denote translocation chromosomes.

pTRS-47 and pTRS-63. Satellite III DNA from chromosome 15 was not detected in case 33, but pTRS-47 from chromosome 14 was retained by the translocation (fig. 6*b*). Both 15q21q translocations contained satellite III DNA. The observed short-arm structures for the translocations involving chromosome 15 are illustrated in figure 7.

Discussion

Previous studies of 13q14q and 14q21q translocations have indicated that the breakpoints in chromosome 14 occur between pTRS-47 and pTRS-63, with satellite I DNA (pTRI-6) usually retained by chromosomes 13 and 21 (Earle et al. 1992; Kalitsis et al. 1993; Han et al. 1994). The results of the present study agree with others, which have narrowed breaks in 13q14q translocations between pTRS-47 and pTRS-63 in chromosome 14. However, our data suggest that 14q21q translocations may be structurally different, on the basis of the presence of chromosome 14 short-arm DNA (fig. 3). The translocations in our study were categorized into one of three structural classes. It should be noted that presently these groups *generally* describe translocation structure; as more repetitive DNA subfamilies are described, the translocations potentially may be categorized into more detailed classes. Only 1 of 10 14q21q translocations did not hybridize with the satellite III DNA probes (class I; case 13), while three others hybridized with both chromosome 14–specific satellite III DNA probes (class III). Still, the majority (six) of the 14q21q translocations, as well as those studied by others reflect a class II structure, in which pTRS-47 but not pTRS-63 is retained (Earle et al. 1992; Kalitsis et al. 1993).

Only two studies have investigated breakpoints in other types of Robertsonian translocations (Cheung et al. 1990; Wolff and Schwartz 1992). A combined nine nonhomologous dicentric Robertsonian translocations that did not include 13q14q or 14q21q translocations were analyzed; the majority of breakpoints occurred distal to the centromeric DNA and proximal to β-satellite DNA and rDNA. In the present study, FISH results of 11 translocations involving chromosome 15 indicated that the structure of these translocations also varies. In most of the 11 translocations (10/11), classical satellite DNA was present (fig. 7, class I and II). Only one case showed that chromosome 15–specific satellite III DNA was absent from the translocation (table 3, case 33; fig. 7, class III).

In earlier studies, we reported nonrandom centromere activity in dicentric Robertsonian translocations and suggested that there is preferential activity of certain centromeres (Sullivan et al. 1994; Sullivan and Schwartz 1995). The chromosome 15 centromere was most frequently the inactive centromere; therefore, we hypothesized that the structure of the translocation might correlate with the inactivity of this centromere, particularly since the only translocation with an active 15 centromere (table 3, case 33) was observed to have no detectable chromosome 15 satellite III DNA (fig. 7, class III). The remaining translocations demonstrated an inactive

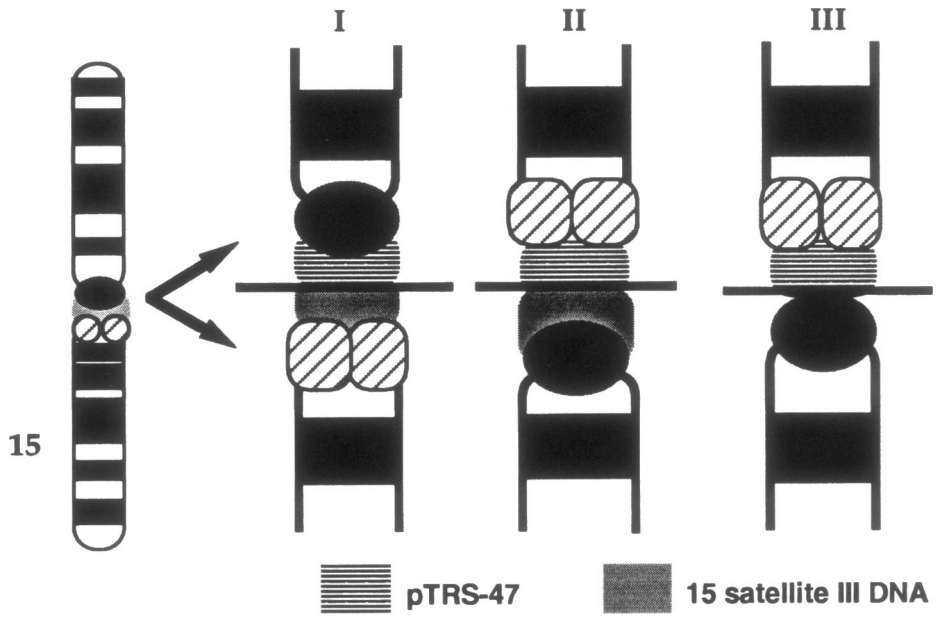

Figure 7 Structural classifications of translocations involving chromosome 15. Most Robertsonian translocations involving chromosome 15 belonged to class I, in which 15-specific satellite III DNA (classical satellite) was present and the 15 centromere was inactive. One case (table 3, case 35) had class II structure (satellite III DNA present, but the 15 centromere *active*), while one case (table 3, case 33) belonged to class III (satellite III DNA absent and 15 centromere *active*). An active centromere is denoted by a dark circle at the primary constriction, and an inactive centromere is represented by two, separated hashed circles.

15 centromere and the presence of satellite III DNA. However, subsequent study of a second 15q21q translocation revealed that the chromosome 15 centromere was active (table 3, case 35) and 15-specific satellite III DNA was present (fig. 7, class II). Therefore, no apparent correlation of centromeric activity with translocation structure was observed, although it is possible that any influence of structure on centromeric activity may be specific to certain translocation types (e.g., t[14q15q] rather than t[15q21q]).

This study describes the short-arm structure of various dicentric Robertsonian translocations, including the largest set of translocations involving chromosome 15 studied to date, and confirms previous reports of the presence of satellite III DNA in 13q14q and 14q21q translocations. These results indicate that satellite III DNA, perhaps specifically the region between pTRS-47 and pTRS-63 and, less often, other short-arm DNAs, predispose acrocentric chromosomes to a high frequency of breakage or recombination events that lead to the formation of Robertsonian translocations. It is unclear whether a specific repetitive DNA, such as satellite III, is predominantly involved in initiating recombination in all or some Robertsonian translocations or whether other repetitive DNAs have important, but indirect, contributions to translocation formation. rDNA is usually not present on the translocations (Brasch and Smyth 1979; Mikkelsen et al. 1980; Cheung et al. 1990; Gravholt et al. 1992; Wolff and Schwartz 1992); however, it may provide the inital point of association for acrocentric short arms, while the unresolved recombination event leading to the formation of a Robertsonian occurs in proximal (satellite III) DNA. Detailed molecular studies in which breakpoints in one or several types of Robertsonian translocations are precisely mapped may identify a specific sequence or motif that directs the formation of these dicentric rearrangements.

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