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DUAL-COLOR FISH ANALYSIS OF BREAKPOINTS ON ROBERTSONIAN TRANSLOCATIONS

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Summary We investigated six Robertsonian translocations, including two cases of rob(13q14q), one of rob(14q21q), one of rob(13q22q), and two of rob(21q21q), by means of fluorescence *in situ* hybridization (FISH) using five repetitive DNA probes: two alpha-satellite DNAs (D21Z1/D13Z1 and D14Z1/D22Z1), satellite III DNA, beta-satellite DNA, and ribosomal DNA. Single color FISH successfully defined the breakpoints in four cases of the six. Since the remaining two cases, rob(13q22q) and rob(21q21q), revealed to retain rDNA, we tried to define the breakpoints in detail by dual color FISH in these rare types. In the rob(13q22q) the chromosomal breakage on chromosome 22 was likely to have occurred within the rDNA region and that the chromosome 13 breakpoint was within the alpha-satellite region. In one rob(21q21q) case we defined the breakpoint on one chromosome distal to, or within, the beta-satellite region distal to the rDNA, and the other chromosome breakage had occurred within alpha-satellite DNA. Our results underscored the power of dual-color FISH for defining the precise locations of breakpoints in Robertsonian translocations.

Key Words Robertsonian translocations, FISH, breakpoints

INTRODUCTION

Robertsonian translocations, whole-arm exchanges between acrocentric chromosomes, are the most common chromosomal rearrangements in humans (Hamerton *et al.*, 1975). Most Robertsonian translocations occur between nonhomologous acrocentric chromosomes (Niebuhr, 1972), and among them rob(13q14q) and rob(14q21q) are the most frequent (Therman *et al.*, 1989). Toward a better understanding of the molecular mechanisms that generate Robertsonian transloca-

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tions, a number of groups have performed FISH analyses to characterize the chromosomal breakpoints (Cheung *et al.*, 1990; Gravholt *et al.*, 1992; Wolff and Schwartz, 1992; Earle *et al.*, 1992; Han *et al.*, 1994; Page *et al.*, 1996). However, those studies have focused mainly on the most common types; rarer types have not been analyzed intensively.

In the present study, using dual-color FISH we characterized the breakpoints in six Robertsonian translocations, including three common types, one less common nonhomologous type, and two involving homologues.

MATERIALS AND METHODS

Cases and DNA probes. Robertsonian translocations observed in six cases are summarized in Table 1. The DNA probes used for FISH analysis were (1) alpha-satellite DNA corresponding to the D21Z1/D13Z1 loci on the centromeres of chromosomes 21 and 13 (Oncor Inc.); (2) alpha-satellite DNA corresponding to the D14Z1/D22Z1 loci on the centromeres of chromosomes 14 and 22 (Oncor Inc.); (3) satellite III DNA synthesized by PCR with primers AATGGAATGGAATGG and CTTTTTCACCTTTTTCAC (Vissel *et al.*, 1992; Dr. M. Ikeno and Dr. H. Masumoto, personal communications); (4) pβ21 plasmid corresponding to beta-satellite, kindly provided by Dr. H.F. Willard (Greig and Willard, 1992); and (5) a cosmid clone, cHKA83, containing rDNA sequence (Kurahashi *et al.*, 1994). The order of these loci on the short arm of the acrocentric chromosomes is as follows: telomere-beta satellite (distal)-rDNA-beta satellite (proximal)-satellite III-alpha satellite-centromere (Gravholt *et al.*, 1992).

Fluorescence in situ hybridization (FISH). The metaphase preparations were made from PHA-stimulated lymphocyte cultures of peripheral blood. FISH experiments were carried out as described previously (Takahashi *et al.*, 1992). Each probe was labeled either with biotin-16-dUTP or with digoxigenin-11-dUTP by nick translation (Boehringer Mannheim). Biotin-labeled probes were detected by avidin FITC and digoxigenin-labeled probes were detected by anti-digoxigenin rhodamine; then chromosomes were counterstained with DAPI (Sigma). FISH signals were visualized with a Nikon Optiphoto fluorescent microscope and superimposed by means of Mac-Probe Ver. 2.5 software (Perceptive Scientific Instruments, Inc., TX).

RESULTS

In metaphase cells from two rob(13q14q) of cases 1 and 2 and from rob(14q21q) of case 3, probes for satellite III and two alpha-satellite of D21Z1/D13Z1 and D14Z1/D22Z1 revealed positive hybridization signals on translocated chromosomes in each case, whereas the beta-satellite and rDNA probes did not hybridize to any of translocated ones (Fig. 1). These results indicated that the

translocated chromosome was dicentric and the breakpoint was located within, or just distal to the satellite III of one chromosome and within, or just proximal to the satellite III region of the other, in all three cases. In case 4 of rob(13q22q), all five probes revealed positive hybridization signals, indicating that the translocated chromosome was also dicentric and the breakpoint on one or both chromosomes lay distal to the rDNA region.

Two rob(21q21q) of cases 5 and 6, each translocated chromosome appeared to be morphologically different in DAPI stained preparation; symmetrical in case 5 and asymmetrical in case 6. FISH analysis revealed that the alpha-satellite probe hybridized to the translocated chromosomes of case 5 but the satellite III probe did not. On the other hand, in case 6 two separated signals for the alpha-satellite probes and a single signal for each of the others (satellite III, beta-satellite, and rDNA) were detected on translocated chromosomes, suggesting that one of the breakpoints was located distal to rDNA. These results are summarized in Table 1.

The breakpoints in rob(13q22q) of case 4 and rob(21q21q) of case 6 were further characterized by dual-color FISH analysis with beta satellite and rDNA. Because the rDNA locates between two beta satellite sequences, these two probes produced tandemly three signals of beta satellite-rDNA-beta satellite on the short arm of normal acrocentrics. The analysis in case 4 of rob(13q22q) using beta-satellite and rDNA probes revealed only a single signal for beta-satellite DNA with rDNA signal in the order cen13-rDNA-beta-satellite-cen22. An additional dual-color FISH analysis with satellite III and rDNA probes also detected a single signal of satellite III corresponding to chromosome 22 (Fig. 2a). These results indicated that the chromosomal breakage on chromosome 22 was likely to have occurred within, or just distal to the rDNA region and, on chromosome 13 within, or just distal to the alpha satellite region (see Fig. 3a).

Dual-color FISH analysis of case 6 showed tandemly arrayed three signals in order of beta satellite-rDNA-beta satellite (Fig. 2b). Satellite III and rDNA probes revealed a single positive signal of satellite III adjacent to the rDNA region (Fig. 2c). These results implied that the breakpoint on one chromosome 21 in this case had occurred distal to, or within the distal beta-satellite region, and that in the other chromosome 21 the breakpoint was located within the alpha-satellite DNA, as illustrated in Fig. 3b.

DISCUSSION

Recent studies using FISH have revealed that most nonhomologous Robertsonian translocations are dicentric (Cheung *et al.*, 1990; Gravholt *et al.*, 1992; Wolff and Schwartz, 1992; Earle *et al.*, 1992; Page *et al.*, 1996). All of the four nonhomologous Robertsonian translocations examined in the present study were dicentric, indicating that the breakage had occurred on the short arms of both acrocentric chromosomes. In the two cases with rob(13q14q) and a case with

Table 1. Karyotypes and FISH results.

Case No.	Karyotype	13/21α	14/22α	Satellite III	β Satellite	rDNA
1	45,XX,rob(13q14q)	+	+	+	−	−
2	45,XX,rob(13q14q)	+	+	+	−	−
3	45,XX,rob(14q21q)	+	+	+	−	−
4	45,XX,rob(13q22q)	+	+	+	+	+
5	46,XX,rob(21q21q)	+	nt.	−	nt.	nt.
6	46,XX,rob(21q21q)	++	nt.	+	+	+

nt., not tested. ++, two separate signals.

rob(14q21q) studied here, our FISH results indicated that their breakpoint on one or both chromosomes was located within satellite III. As the satellite III probe we used was not chromosome-specific, this observation was not conclusive, but it is likely that the chromosomal breakage on both chromosomes occurred within the satellite III DNA. Our results are consistent with a number of previous reports that located breakpoints of rob(13q14q) and rob(14q21q) within satellite III DNA (Gravholt *et al.*, 1992; Han *et al.*, 1994; Page *et al.*, 1996).

Our experiments showed that the breakpoints of rob(13q22q) were located within the rDNA region on chromosome 22 and the alpha-satellite DNA on chromosome 13. Chromosomal breakpoints among rare types of nonhomologous Robertsonian translocations occur in various regions (Page *et al.*, 1996). Studies using silver staining have revealed that most Robertsonian translocations occur in conjunction with deletion of the nucleolar organizing region (NOR) (Brasch and Smyth, 1979; Mattei *et al.*, 1979; Mikkelsen *et al.*, 1980). Page *et al.* (1996) revealed by FISH analysis that the rDNA region was retained in only one among the 56 Robertsonian chromosomes they examined. Therefore, the chromosome translocation we observed in case 4 is considered to be a very rare type.

Most of the homologous Robertsonian translocations so far reported have been monocentric (Wolff and Schwartz, 1992) and isochromosomes (Grasso *et al.*, 1989; Antonarakis *et al.*, 1990; Shaffer *et al.*, 1991). Of the two cases of homol-

Fig. 1. FISH on metaphase chromosomes of case 3, rob(14q21q) using rDNA as the probe. Only 8 positive signals were detected (arrowheads), suggesting that rDNA was deleted in the translocation chromosomes.

Fig. 2. (a) Dual-color FISH with satellie III and rDNA probes on metaphase chromosomes of case 4, rob(13q22q). Each positive signal of satellite III and rDNA is observed, in the order chromosome 13-rDNA (red)-satellite III (green)-chromosome 22. (b) Dual-color FISH with beta-satellite and rDNA on metaphase chromosomes of case 6, rob(21q21q). Hybridization signals of beta-satellite (green) are seen on both sides of rDNA (red). (c) Dual-color FISH with satellite III and rDNA probes on metaphase chromosomes of case 6. One satellite III signal (green) is present beside the rDNA (red).

Fig. 3. Possible translocated chromosomes in cases 4 (a) and 6 (b). The order of these repetitive DNA loci on the short arm of the normal acrocentric chromosomes is telomere-beta satellite (red)-rDNA (yellow)-beta satellite (red)-satellite III (green)-alpha satellite (blue)-centromere. Lines with arrowheads indicate breakpoints and exchanges for the involved acrocentrics in each case.

Fig. 1.

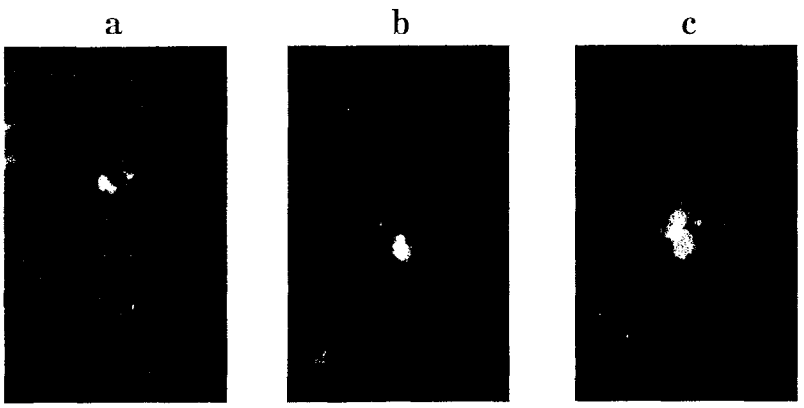

Fig. 2.

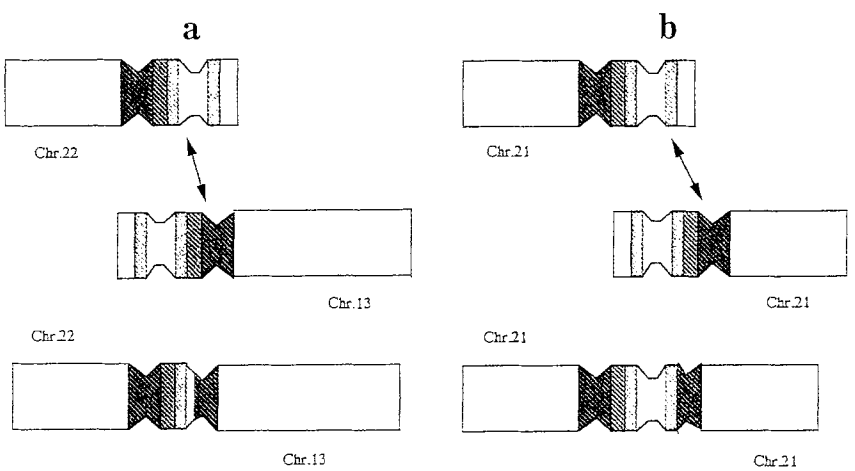

Fig. 3

ogous Robertsonian translocation studied here, we believe one to be monocentric and the other to be dicentric with retention of the rDNA region, similar to the rob(13q22q) of case 4. Hence, the translocated chromosome of case 6 is not an isochromosome and it also constitutes a very rare type.

As represented by the analysis of cases 4 and 6, single-color FISH did not permit us to distinguish the two chromosomes from which the signals were derived, while dual-color FISH clarified the precise location of the breakpoints. Hence, our results indicate that dual-color FISH is a powerful tool for defining locations of the breakpoints in Robertsonian translocations.

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REFERENCES

Antonarakis SE, Adelsberger PA, Petersen MB, Binkert F, Schinzel AA (1990): Analysis of DNA polymorphisms suggests that most *de novo* dup(21q) chromosomes in patients with Down syndrome are isochromosomes and not translocations. Am J Hum Genet **47**: 968-972

Brasch JM, Smyth DR (1979): Absence of silver bands in human Robertsonian translocation chromosomes. Cytogenet Cell Genet **24**: 122-125

Cheung SW, Sun L, Featherstone T (1990): Molecular cytogenetic evidence to characterize breakpoint regions in Robertsonian translocations. Cytogenet Cell Genet **54**: 97-102

Earle E, Shaffer LG, Kalitsis P, McQuillan C, Dale S, Choo KHA (1992): Identification of DNA sequences flanking the breakpoint of human t(14q21q) Robertsonian translocations. Am J Hum Genet **50**: 717-724

Grasso M, Giovannucci UML, Tavellini F, Perroni L, Dagna BF (1989): Isochromosome not translocation in trisomy 21q21q. Hum Genet **84**: 63-65

Gravholt CH, Friedrich U, Caprani M, Jorgensen AL (1992): Breakpoints in Robertsonian translocations are located to satellite III DNA by fluorescence *in situ* hybridization. Genomics **14**: 924-930

Greig GM, Willard HF (1992): β-Satellite DNA: Characterization and localization of two subfamilies from the distal and proximal short arms of the human acrocentric chromosomes. Genomics **12**: 573-580

Hamerton JL, Canning N, Ray M, Smith S (1975): A cytogenetic survey of 14,069 newborn infants. I. Incidence of chromosome abnormalities. Clin Genet **8**: 223-243

Han JY, Choo KHA, Shaffer LG (1994): Molecular cytogenetic characterization of 17 rob(13q14q) Robertsonian translocations by FISH, narrowing the region containing the breakpoints. Am J Hum Genet **55**: 960-967

Kurahashi H, Akagi K, Karakawa K, Nakamura T, Dumanski JP, Sano T, Okada S, Takai S, Nishisho I (1994): Isolation and mapping of cosmid markers on human chromosome 22, including one within the submicroscopically deleted region of DiGeorge syndrome. Hum Genet **93**: 248-254

Mattei MG, Mattei JF, Ayme S, Giraud F (1979): Dicentric Robertsonian translocations in man: 17 cases studied by R, C, and N banding. Hum Genet **26**: 14-21

Mikkelsen M, Basli A, Poulsen H (1980): Nucleolus organizer regions in translocations involving acrocentric chromosomes. Cytogenet Cell Genet **26**: 14-21

Niebuhr E (1972): Dicentric and monocentric Robertsonian translocations in man. Humangenetik **16**: 217-226

Page SL, Shin JC, Han JY, Choo KHA, Shaffer IG (1996): Breakpoint diversity illustrates

distinct mechanisms for Robertsonian translocation formation. Hum Mol Genet **5**: 1279–1288

Shaffer LG, Jackson CCK, Mayer JM, Brown JA, Spence JE (1991): A molecular genetic approach to the identification of isochromosomes of chromosome 21. Hum Genet **86**: 375–382

Takahashi E, Yamakawa K, Nakamura Y, Hori T (1992): A high-resolution cytogenetic map of human chromosome 3: Localization of 291 new cosmid markers by direct R-banding fluorescence *in situ* hybridization. Genomics **13**: 1047–1055

Therman E, Susman B, Denniston C (1989): The nonrandom participation of human acrocentric chromosomes in Robertsonian translocations. Ann Hum Genet **53**: 49–65

Vissel B, Nagy A, Choo KH (1992): A satellite III sequence shared by human chromosomes 13, 14 and 21 that is contiguous with alpha satellite DNA. Cytogenet Cell Genet **61**: 81–86

Wolff DJ, Schwartz S (1992): Characterization of Robertsonian translocations by using fluorescence *in situ* hybridization. Am J Hum Genet **50**: 174–181