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Identification of DNA Sequences Flanking the Breakpoint of Human t(14q21q) Robertsonian Translocations

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Summary

We have employed molecular probes and in situ hybridization to investigate the DNA sequences flanking the breakpoint of a group of t(14q21q) Robertsonian translocations. In all the families studied, the probands were patients with Down syndrome who carried a de novo t(14q21q) translocation. The DNA probes used were two alphoid sequences, alphaRI and alphaXT, which are specific for the centromeres of chromosomes 13 and 21 and of chromosomes 14 and 22, respectively; a satellite III sequence, pTRS-47, which is specific for the proximal p11 region of chromosomes 14 and 22; and a newly defined satellite III DNA, pTRS-63, which is specific for the distal p11 region of chromosome 14. The two alphoid probes detected approximately the same amount of autoradiographic signal on the translocated chromosomes as was expected for chromosomes 14 and 21 of the originating parent, suggesting that there has been no loss of these centromeric sequences during the translocation events. Results with the two satellite III probes indicated that the domain corresponding to pTRS-47 was retained in the translocated chromosomes, whereas the domain for pTRS-63 was lost. These results have allowed us to place the translocation breakpoint between the pTRS-47 and pTRS-63 domains within the p11 region of chromosome 14.

Introduction

Robertsonian translocations are the most commonly observed chromosomal rearrangement in man (Jacobs et al. 1974; Hook and Hamerton 1977). These translocations generally involve the exchange of whole chromosome arms between two nonhomologous acrocentric chromosomes. Such an exchange results in one chromosome being formed by the two long (i.e., q) arms and in a second chromosome being formed by the two short (i.e., p) arms. The latter chromosome is usually acentric and will be lost in subsequent cell divisions.

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Robertsonian translocations involving all combinations of the five different acrocentric chromosomes have been observed. However, an unusual feature of these translocations is that they are highly nonrandom, with exchanges between chromosomes 13 and 14 and between 14 and 21 together constituting 65%–80% of all cases (Therman 1986; Therman et al. 1989). Robertsonian translocations involving chromosome 21 are responsible for about 5% of patients with Down syndrome (Hamerton 1981).

The etiological mechanisms underlying Robertsonian translocations are presently not clear. As a step toward a molecular understanding of such mechanisms, we have used different cloned DNA probes that have been mapped to the centromere or p-arm regions of the acrocentric chromosomes, to characterize a number of families in whom a de novo translocation involving chromosomes 14 and 21 had occurred. The results have allowed us to narrow down the chromosomal region that contains the breakpoint for the t(14q21q) group of Robertsonian translocations.

Subjects and Methods

Robertsonian Translocation Families

The following unrelated families were used in the present study: T8954, T9001, T9003, T9004, T9007, T9012, T9013, T9014, T9016, T9019, T9023, and T9025. In all cases, the family members included both parents and one proband who has Down syndrome due to a de novo t(14q21q) Robertsonian translocation chromosome additional to the two normal chromosomes 21 and to a single normal chromosome 14. Except for families T9003, T9019, and T9023, the translocations have been shown to be maternal in origin, by a combination of RFLP (using D21S13, D21S16, D21S52, D21S59, D21S26, D21S24, D21S15, D21S19, and D21S113 as probes) and chromosomal heteromorphism testing; T9003 and T9023 were considered to be maternally derived on the basis of chromosomal heteromorphism only, whereas parental origin could not be determined in T9019 (Shaffer et al., in press). Transformed lymphocyte cultures were established from all the individuals and were karyotyped at the Medical College of Virginia; they were rekaryotyped at the Murdoch Institute before they were used in the present study.

DNA Probes

The DNA probes used were (1) alphaRI (Jorgensen et al. 1987), (2) alphaXT (Jorgensen et al. 1988), (3) pTRS-47 (Choo et al. 1990), and (4) pTRS-63 (Choo et al. 1992).

In Situ Hybridization

The procedure for in situ hybridization was according to a method described elsewhere (Choo et al. 1991*a*). In brief, metaphase chromosomes were hybridized with ^{3}H-labeled probes, followed by banding using the bromodeoxyuridine-incorporation method, to allow direct visualization of autoradiographic grains on chromosome bands. After hybridization, the final washing stringency used for the various probes was 0.1 × SSC at 65°C. In these analyses, slides from individuals in a family were hybridized and treated identically within the same experiment, to allow direct comparison of autoradiographic signals.

Results

In a separate study (Shaffer et al., in press) the parental origin of the t(14q21q) translocations in the different families was determined. Since in most cases this was shown to be maternal, by a combination of RFLP testing and cytogenetic heteromorphism, in the present study we have presented results only for the maternal chromosomes. Where the parental origin was either (*a*) not established because of uninformative RFLPs and cytogenetic heteromorphisms (family T9019) or (*b*) incompletely established (families T9003 and T9023; maternal origin established on the basis of cytogenetic heteromorphism only), results for both parents are shown.

Analysis with pTRS-47 Probe

pTRS-47 is a satellite III DNA subfamily which has previously been demonstrated on the proximal p-arm region of human chromosome 14 (also on chromosome 22; Choo et al. 1990). In the present study, we have used this probe to analyze the different Robertsonian translocation chromosomes. In all the families looked at, strong hybridization signals similar in intensity to those seen on normal chromosomes 14 were detected on the translocated chromosomes (fig. 1). This observation was confirmed by direct scoring of autoradiographic grains (data not shown). These results therefore indicated that the pTRS-47 domain was largely if not fully (see Discussion) retained on the translocated chromosomes.

Analysis with pTRS-63 Probe

pTRS-63 is a newly isolated satellite III probe that is specific for the p arm of human chromosome 14 (Choo et al. 1992). In the present study, the probe was used for in situ hybridization analysis of the different Robertsonian translocation families. Direct examination of the chromosomes revealed that the amount of signal on the translocated chromosomes was significantly lower than that found on the chromosomes 14 of the originating parent. Figure 2 shows results for the distribution of autoradiographic grains on the relevant chromosomes (i.e., chromosomes 14 and 21 of the originating parent, and chromosome t(14q21q) of the proband). However, since we did not know which of the two homologues of chromosomes 14 and 21 in the parent concerned were involved in the translocation event, we have used the sum of the average values for each pair of homologues to derive the expected grain count for a translocation product in which there was no loss of sequences corresponding to the pTRS-63 probe. The results in figure 2 (I–X) clearly indicate that in all the t(14q21q) families analyzed (including the two where parental origin was not certain), the observed hybridization signals on the translocated

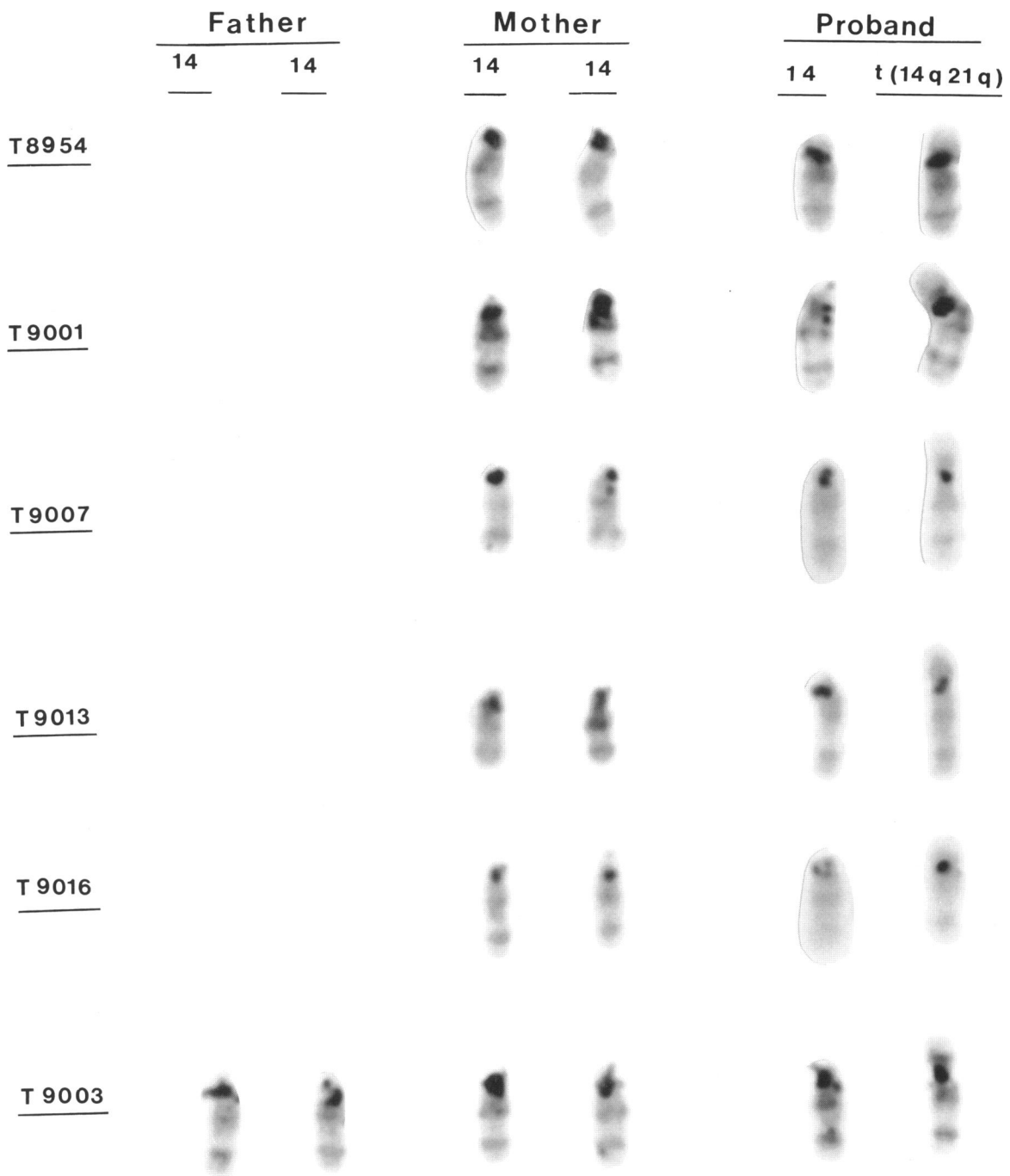

Figure 1 In situ hybridization using pTRS-47 probe. The t(14q21q) translocation chromosome and the normal chromosomes 14 of the father, mother, and proband are shown. In families T8954, T9001, T9007, T9013, and T9016, the translocations are known to be maternal in origin, and therefore only the maternal chromosomes 14 are shown. In family T9003, since the parental origin of the translocation was not certain, chromosomes 14 from both the mother and the father are shown. The varying amounts of hybridization signals seen in the different families were related to the different autoradiographic exposure times used, although exposure time for individuals within a family was kept constant.

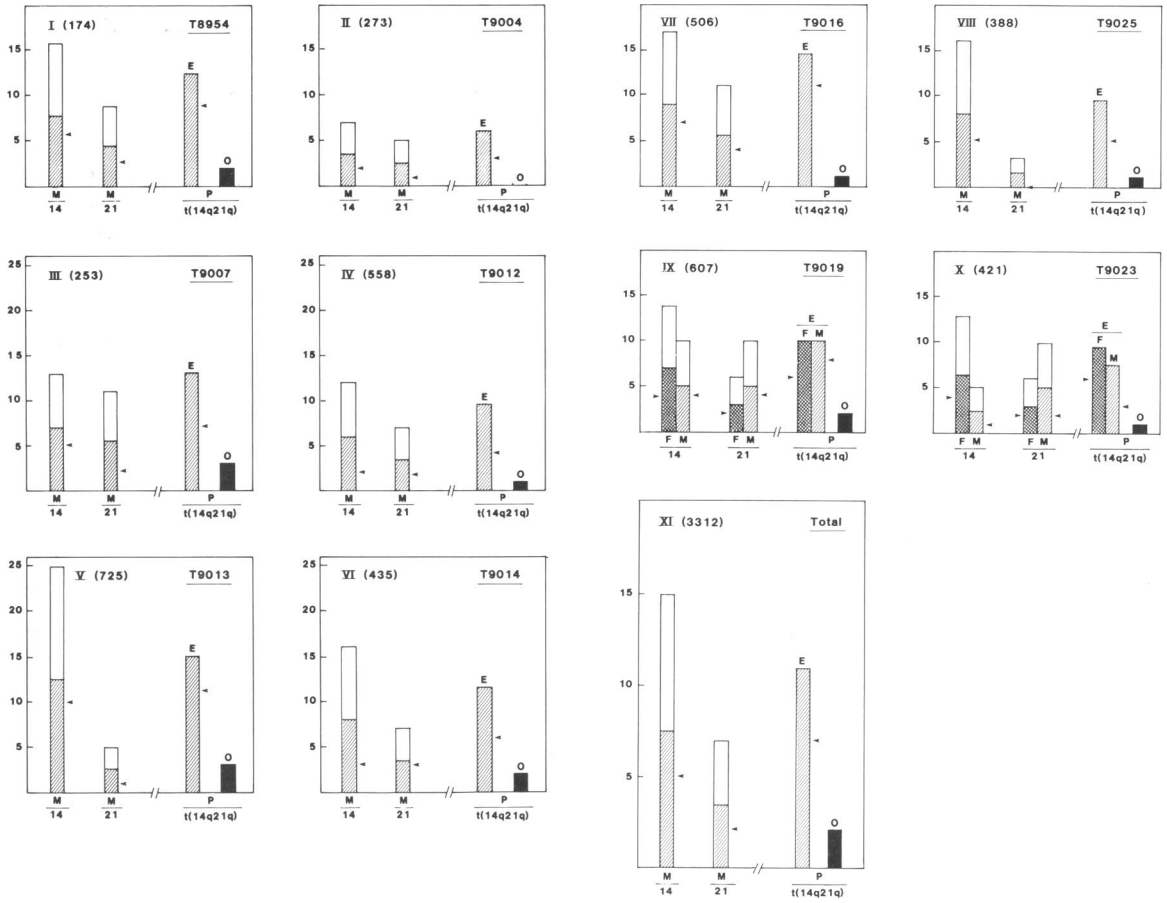

Figure 2 In situ hybridization of t(14q21q) Robertsonian translocation families by using pTRA-63 probe. The vertical axis shows the counts of autoradiographic grains over chromosomes 14 and 21 or over the translocated chromosome. The horizontal axis indicates chromosomes 14 and 21 derived from mother (M) or father (F) and the t(14q21q) chromosome derived from the proband (P). I–VIII are families in whom the translocations were known to be maternal in origin, whereas IX and X are families in whom the parental origin was uncertain. XI represents the sum of the results shown in I–VIII. The actual number of autoradiographic grains counted are shown in parenthesis, but these are normalized to 100 in the histograms. O = observed grain counts on translocated chromosomes; and E = grain count expected for the t(14q21q) chromosome (value is based on either the average value [hatched or cross-hatched bar] or the lower of the two values (arrow) for each of the pair of maternal [or paternal] homologues for chromosomes 14 and 21).

chromosomes were significantly lower than the expected values. This trend was reflected in the pooled data for the eight families in whom the maternal origin of the translocated chromosome was certain (fig. 2, XI). If, instead of taking the average grain counts to derive the expected value for the translocated chromosome, we conservatively assume that, for every cell scored, the chromosomes 14 and 21 participating in the translocation have the lower grain count of the two homologues (fig. 2, values indicated by arrows), then the observed value is still significantly lower than this "lowest possible" expected value. These results therefore provide good evidence that the pTRS-63 domain on the p arm of the participating chromosome 14 was largely, if not totally (see Discussion), lost in the translocation event.

Analysis with alphaRI and alphaXT Probes

Alpha RI and alpha XT are two alpha satellite DNA probes that hybridize preferentially to the centromeric regions of human chromosomes 13 and 21 and of chromosomes 14 and 22, respectively (Jorgensen et al. 1987, 1988). Figure 3 (I–V) shows in situ hybridization results obtained with five different Robertsonian translocation families. As can be seen, with both the

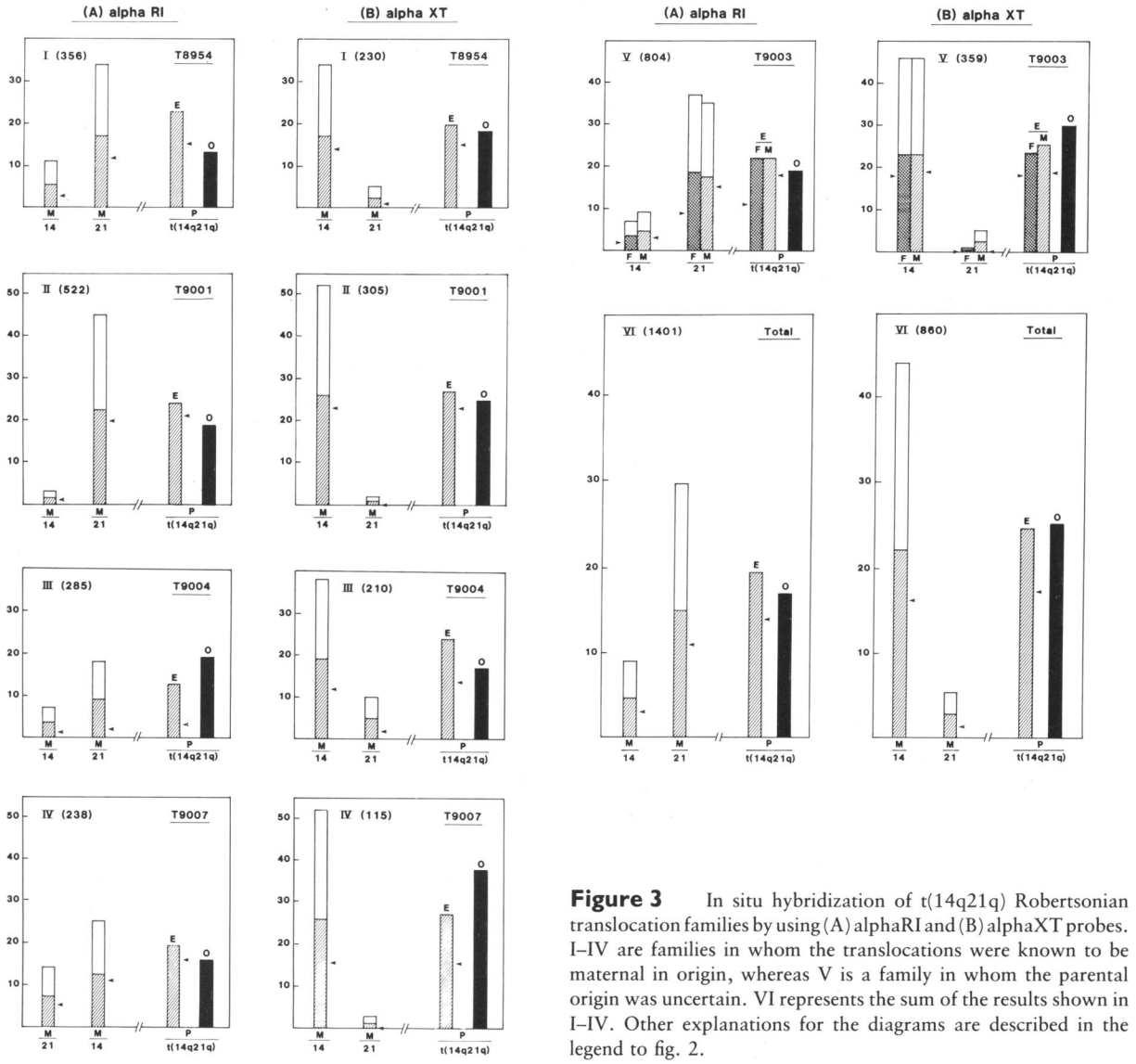

Figure 3 In situ hybridization of t(14q21q) Robertsonian translocation families by using (A) alphaRI and (B) alphaXT probes. I–IV are families in whom the translocations were known to be maternal in origin, whereas V is a family in whom the parental origin was uncertain. VI represents the sum of the results shown in I–IV. Other explanations for the diagrams are described in the legend to fig. 2.

probes a substantial amount of autoradiographic signal was observed on all the t(14q21q) chromosomes, an amount which was quantitatively similar to the levels expected for a translocation event in which there was no loss of sequences corresponding to the probes used. By considering the four families as a group in whom the maternal origin was certain, the signal observed on the translocated chromosomes proved to be closely similar to that expected (fig. 3, VI). If, instead of taking the average grain counts to derive the expected value for the translocated chromosome, we assume that, for every cell scored, the chromosomes 14 and 21 participating in the translocation have the lower grain count of the two homologues (fig. 3, values indicated by arrows), then the observed values turn out, in most cases, to be similar if not slightly higher than the expected values. Besides the five families shown in figure 3, four other families (T9012, T9013, T9014, and T9016) have been similarly analyzed by using the alphaRI and alphaXT probes, with same results (data not shown). On the basis of this evidence, it can be concluded that the two centromeric domains defined by alphaRI on chromosome 21 and by alphaXT on chromosome 14 have been retained and have produced an apparently dicentric t(14q21q) chromosome.

Discussion

In previous studies the precise localization of the chromosomal site of Robertsonian translocation has been difficult because of (*a*) limitation in the resolution of cytogenetic banding techniques, (*b*) extensive size heteromorphism of the cen-pter regions of the acrocentric chromosomes, and (*c*) the lack of a good molecular map of these regions. Recent isolation of clearly distinct families or subfamilies of alpha satellite, satellite III, and other types of repetitive DNA from the acrocentric chromosomes should provide useful probes for the detailed characterization of the sequences that are directly involved in the translocation events (for reviews, see Choo 1990, Choo et al. 1991*b*; also see Choo et al. 1990, 1992). The present study utilized a number of these probes in the analysis of the t(14q21q) group of Robertsonian translocations.

There are two difficulties associated with our analysis. First, under the stringency of the in situ hybridization conditions used, the different satellite DNA probes show some cross-hybridization to closely related sequences. (With these probes, our attempts to raise the stringency of in situ hybridization, to achieve greater specificity, have been unsuccessful, as this only led to a nonspecific removal of all signals from the slides). The second difficulty relates to our inability to cytogenetically distinguish between members of each of the pair of homologues for chromosomes 14 and 21 in the originating parent. This latter information is relevant because of the quantitative polymorphisms that are commonly associated with these satellite sequences (Jorgenson et al. 1987, 1988; Choo et al. 1990, 1992). These difficulties have made it impossible for us to derive absolutely quantitative data. However, despite these limitations, the data obtained in the present study have allowed us to identify candidate sequences that flank both sides of the translocation breakpoint.

The results with alphaRI and alphaXT probes indicate that in all the families studied, there was no significant loss of hybridization signal on the translocated chromosomes, compared with that present on the original chromosomes 14 and 21. These results suggest that the centromeric domains corresponding to the alphaRI sequences on chromosome 21 and to the alphaXT sequences on chromosome 14 have been retained with the long arms in forming the translocated chromosomes.

Earlier cytogenetic studies (Mikkelsen et al. 1980; Schmickel et al. 1985) have indicated that the site of the t(14q21q) translocations is proximal to the nucleolar organizing region. In a single case of t(14q21q) translocation, Cheung et al. (1990) have presented in situ hybridization evidence suggesting that the breakpoint is between the ribosomal gene cluster and the centromeric alpha satellite DNA. Here, we have employed two newly isolated satellite III sequences (pTRS-47 and pTRS-63) to further define the breakpoint region. These sequences have previously been mapped to the p11 region of chromosome 14, where pTRS-63 is located more distal to pTRS-47 (Choo et al. 1990, 1992). Results obtained in the present study indicate that the domain corresponding to pTRS-47 is largely retained on the translocated chromosomes, whereas the domain for pTRS-63 is largely lost. These results therefore allow us to place the translocation breakpoint between these two domains. It is possible that the breaks may have occurred either (*a*) at the very distal end but still within the pTRS-47 domain or (*b*) at the very proximal end but still within the pTRS-63 domain. We believe these possibilities to be unlikely and favor the possibility that the translocations actually take place outside of these two satellite III subfamilies, within an as yet undefined domain (see below). On the basis of the results of the present study, the karyotype of the t(14q21q) group of Robertsonian translocations that we have examined can be designated as either 46,XX or XY,-14, + dic(14;21)(p11; p11).

To date, the best model that has been put forward to explain the frequent occurrence of the t(14q21q) Robertsonian translocations is that the centromere–p arm regions of chromosomes 14 and 21 share a common domain of sequence homology, which is inverted on one of these chromosomes (Ferguson-Smith 1967; Schmickel et al. 1985; Guichaoua et al. 1986; Therman et al. 1989; Choo 1990); a recombinational exchange within this homologous region would then lead to a translocation of the q arms. On the basis of this model, neither pTRS-47 nor pTRS-63 can be the candidate, since one is shared by chromosomes 14 and 22 while the other is specific for chromosome 14 only. We have previously described a group of alpha satellite subfamilies that are shared by chromosomes 14 and 21 (also by chromosome 13) and have suggested these to be possible candidate sequences for the translocation breakpoint (Choo et al. 1988; Choo 1990; Vissel and Choo 1991). More recent studies (Hills et al. 1991; Choo et al. 1992) indicating the absence of alpha satellite sequences between the pTRS-63 and pTRS-47 domains on at least some chromosomes 14

have made the described 13/14/21 sequences unlikely to be involved. This does not, however, exclude the possibility that some chromosomes 14 may carry a pericentric inversion which brings the 13/14/21 alpha satellite domain in between pTRS-63 and pTRS-47 and are therefore predisposed to Robertsonian translocations. Further studies will be needed to clarify these possibilities.

The study of the nature of the breakpoints in Robertsonian translocations is important, as it not only allows us to gain a direct understanding of the etiological mechanism specifically responsible for these translocations but also allows us to more generally define the overall molecular "forces" that govern the way in which the different nonhomologous acrocentric chromosomes interact with one another. Such interactions have previously been thought to be mainly mediated by interchromosomal recombination between rDNA genes within the p12 regions of these chromosomes (Arnheim et al. 1980; Krystal et al. 1981; Schmickel et al. 1985). The identification of clearly definable alpha satellite and satellite III DNA families and subfamilies that show properties of complex interchromosomal sharing within the centromeres and the p arms suggests a potential role of these sequences in determining the interactive behavior of these acrocentric chromosomes (for reviews, see Choo 1990; Choo et al. 1991*b*; also see Choo et al. 1990, 1992). It is conceivable that some of the interactions may lead to meiotic errors such as chromosomal translocations and nondisjunctions (Choo 1990). As a step toward understanding such behavior, we have investigated the nature of the sequences surrounding the t(14q21q) group of Robertsonian translocations. Although we have not defined the precise translocation breakpoint, the identification of pTRS-47 and pTRS-63 as the closest flanking sequences is a significant step toward this goal. It is also pertinent that the probes and the general strategy used in these studies should be readily applicable to the investigation of the breakpoint found in the other common type of Robertsonian translocation: t(13q14q).

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