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A Common Breakpoint on 11q23 in Carriers of the Constitutional t(11;22) Translocation

L. Edelmann,[1] E. Spiteri,[1] N. McCain,[1] R. Goldberg,[1] R. K. Pandita,[1] S. Duong,[1] J. Fox,[2] D. Blumenthal,[2] S. R. Lalani,[3] L. G. Shaffer,[3] and B. E. Morrow[1]

[1]Department of Molecular Genetics, Albert Einstein College of Medicine, Bronx, New York; [2]Schneider Children's Hospital, Long Island Jewish Medical Center, New Hyde Park, New York; and [3]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston

Summary

Structural chromosomal rearrangements occur commonly in the general population. Individuals that carry a balanced translocation are at risk of having unbalanced offspring; therefore, the frequency of translocations in couples with recurrent spontaneous abortions is higher than that in the general population. The constitutional t(11;22) translocation is the most common recurrent non-Robertsonian translocation in humans and may serve as a model to determine the mechanism that causes recurrent meiotic translocations. We previously localized the t(11;22) translocation breakpoint to a region on 22q11 within a low-copy repeat, termed "LCR22." To define the breakpoint on 11q23 and to ascertain whether this region shares homology with LCR22 sequences, we performed haplotype analysis on patients with der(22) syndrome. We found that the breakpoint on 11q23 occurred between two genetic markers, D11S1340 and APOC3-tetra, both being present within a single bacterial-artificial-chromosome clone. To determine whether the breakpoint occurred within the same region among a larger set of carriers, we performed FISH mapping studies. The breakpoints were all within the same clone, suggesting that this region may harbor sequences that are prone to breakage. We narrowed the breakpoint interval, in both derivative chromosomes from two unrelated carriers, to a 190-bp, AT-rich repeat, which indicates that this repeat may mediate recombination events on chromosome 11. Interestingly, the LCR22s harbor AT-rich repeats, suggesting that this sequence motif may mediate recombination events in nonhomologous chromosomes during meiosis.

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Address for correspondence and reprints: Dr. B. E. Morrow, Department of Medical Genetics, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, New York, 10461. E-mail: morrow@aecom.yu.edu

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Introduction

Carriers of the constitutional t(11;22) translocation are at risk of having offspring with a severe congenital anomaly disorder referred to as "der(22) syndrome due to 3:1 meiotic nondisjunction events" (Fraccaro et al. 1980; Zackai and Emanuel 1980). Patients with der(22) syndrome carry a supernumerary der(22) chromosome and are therefore trisomic for 11q23-qter and 22pter-q11. The main clinical findings of der(22) syndrome are moderate mental retardation, mild craniofacial anomalies, and congenital heart defects (Zackai and Emanuel 1980; Lin et al. 1986). To determine the molecular basis of the reciprocal t(11;22) translocation in the normal carrier parents, it is necessary to characterize the breakpoint intervals on 11q23 and 22q11.

The 22q11 region is also susceptible to rearrangements associated with velocardiofacial syndrome/DiGeorge syndrome (VCFS [MIM 192430]; DGS [MIM 188400]) and cat-eye syndrome (CES [MIM 115470]). Patients with VCFS/DGS have hemizygous deletions of part of 22q11. Most cases occur sporadically in the population, suggesting that this region is prone to chromosome breakage. More than 90% of patients were found to have a similar 3-Mb deletion, 7% had a nested distal deletion breakpoint resulting in a 1.5-Mb deletion, and a few rare patients had unique deletions (Carlson et al. 1997). Physical-mapping studies were performed to identify sequences that could confer susceptibility to chromosome deletions. A low-copy repeat that was >200 kb in size and that contained a set of genes or pseudogenes was discovered (Edelmann et al. 1999*a*). The deletions are generated by both inter- and intrachromosomal homologous recombination events between LCR22 sequences (Baumer et al. 1998; Edelmann et al. 1999*a*).

Patients with CES are characterized by ocular colobomata, anal atresia, craniofacial anomalies, and mild mental retardation, and they carry a supernumerary bisatellited chromosome 22 containing the 22pter-q11 region (Schinzel et al. 1981; Reiss et al.

1985; Mears et al. 1994, 1995). Two duplication breakpoints—a smaller CESI and a larger CESII—were identified (McTaggart et al. 1998). These breakpoints occurred in the same two LCR22s, proximal and distal, respectively, that were seen for the common 3-Mb VCFS/DGS deletion breakpoints (Edelmann et al. 1999*b*). Further evidence that the LCR22 sequences mediate chromosome rearrangements came from haplotype and FISH analysis of a family that carried an interstitial duplication of the 3-Mb region (Edelmann et al. 1999*b*). An interchromosomal homologous recombination event between the two LCR22s mediated the 3-Mb duplication (Edelmann et al. 1999*b*). These results demonstrate that the LCR22s mediate different rearrangements on 22q11, leading to different congenital anomaly disorders.

To define the t(11;22) breakpoint on 22q11, haplotype analysis, FISH, and physical-mapping studies were performed on patients with der(22) syndrome and their carrier parents (Funke et al. 1999). The t(11;22) breakpoint occurred in the same LCR22 as it did for the distal 1.5-Mb deletion breakpoint in patients with VCFS/DGS (Edelmann et al. 1999*b*; Funke et al. 1999). Therefore, there are three regions on 22q11 that are prone to breakage; they are 1.5 Mb apart, and each contains an LCR22. In this report, we defined the region that contains the t(11;22) breakpoint on 11q23 to determine whether subsets of LCR22 sequences lie within the interval.

Patients and Methods

Patients

Carriers of the t(11;22) translocation and patients with der(22) syndrome were ascertained and diagnosed with the use of "BM" (Bernice Morrow) codes, as described elsewhere (Funke et al. 1999). Cell lines from the t(11;22) translocation carriers GM06229B, GM04403, GM07332, GM03847, GM03372, and GM06275A and from der(22) syndrome patients GM06228, GM04370A, GM07331, and GM00084A were purchased from the National Institute of General Medical Sciences (NIGMS) cell repository (Coriell Cell Repositories).

Genotype Analysis

Each sample containing 100 ng genomic DNA was genotyped separately with eight different genetic markers—D11S1992, D11S4145, D11S1885, D11S965, D11S1340, APOC3-tetra, D11S4127, and D11S1299—that span the 11q23 region. D11S markers (Research Genetics) were purchased. The APOC3-tetra genetic marker was designed from the genomic sequence in the third intron of the *Apolipoprotein C3* gene (GenBank accession number J00098). The genetic marker examined the status of the same tetranucleotide repeat (CTTT), as described elsewhere (Bhattacharya et al. 1991). For genotyping, one of two primers was radiolabeled with $\gamma[^{32}P]$-ATP, and a PCR product was amplified under standard reaction conditions (Morrow et al. 1995). The radiolabeled PCR products were separated on 6% acrylamide denaturing sequencing gels, and alleles were assigned according to their molecular weight. Proper Mendelian inheritance of each marker was manually confirmed. Genotype analysis using genetic markers on 22q11 was performed as described elsewhere (Carlson et al. 1997; Funke et al. 1999).

Isolation of Bacterial-Artificial-Chromosome (BAC) Clones

To construct a high-resolution physical map of the interval that harbors D11S1340 and APOC3-tetra, high-density gridded membranes containing the 21.8X, RPCI-13 BAC library (BACPAC Resource Center, Department of Cancer Genetics, Roswell Park Cancer Institute) were screened. The probe used for screening was developed by radiolabeling a PCR product from the *APOA1* gene on 11q23 with $\alpha[^{32}P]$-dCTP (Rediprime labeling system; Amersham). The primers used to amplify part of *APOA1* are 5′-CTGAGCCGAAAGGCCAAGCTTGG-3′ and 5′-TGCCCCAGGCCGGGCCTCTGG-3′ (GenBank accession number J00098). Genomic DNA served as a template for the PCR reactions. The positive clones were isolated, and DNA was prepared from bacterial cultures derived from purified colonies (Qiagen). The marker content of each clone was verified by PCR analysis with 10 ng template DNA used with standard amplification conditions (PE Biosystems).

Somatic-Cell Hybrid Cell Lines

The method used to generate hamster-human somatic-hybrid cell lines from t(11;22) carriers BM114 and GM 06229B has been described elsewhere (Carlson et al. 1997). In brief, polyethylene glycol (EM Science) was used to mediate cell fusion of Epstein-Barr virus–transformed lymphoblastoid cells from the patients with hypoxanthine-guanine phosphoribosyltransferase–deficient Chinese-hamster ovary fibroblast CHTG49 cells. Individual clones were tested by PCR for retention of chromosomes X, 11, and 22. The selection for chromosome X is necessary, since there is no efficient positive selection system available for retention of chromosome 11 or chromosome 22. The positive clones containing chromosome 11 and chromosome 22 were expanded and genotyped with genetic markers spanning the chromosome 11q23 and 22q11 region, to confirm the integrity and identity of

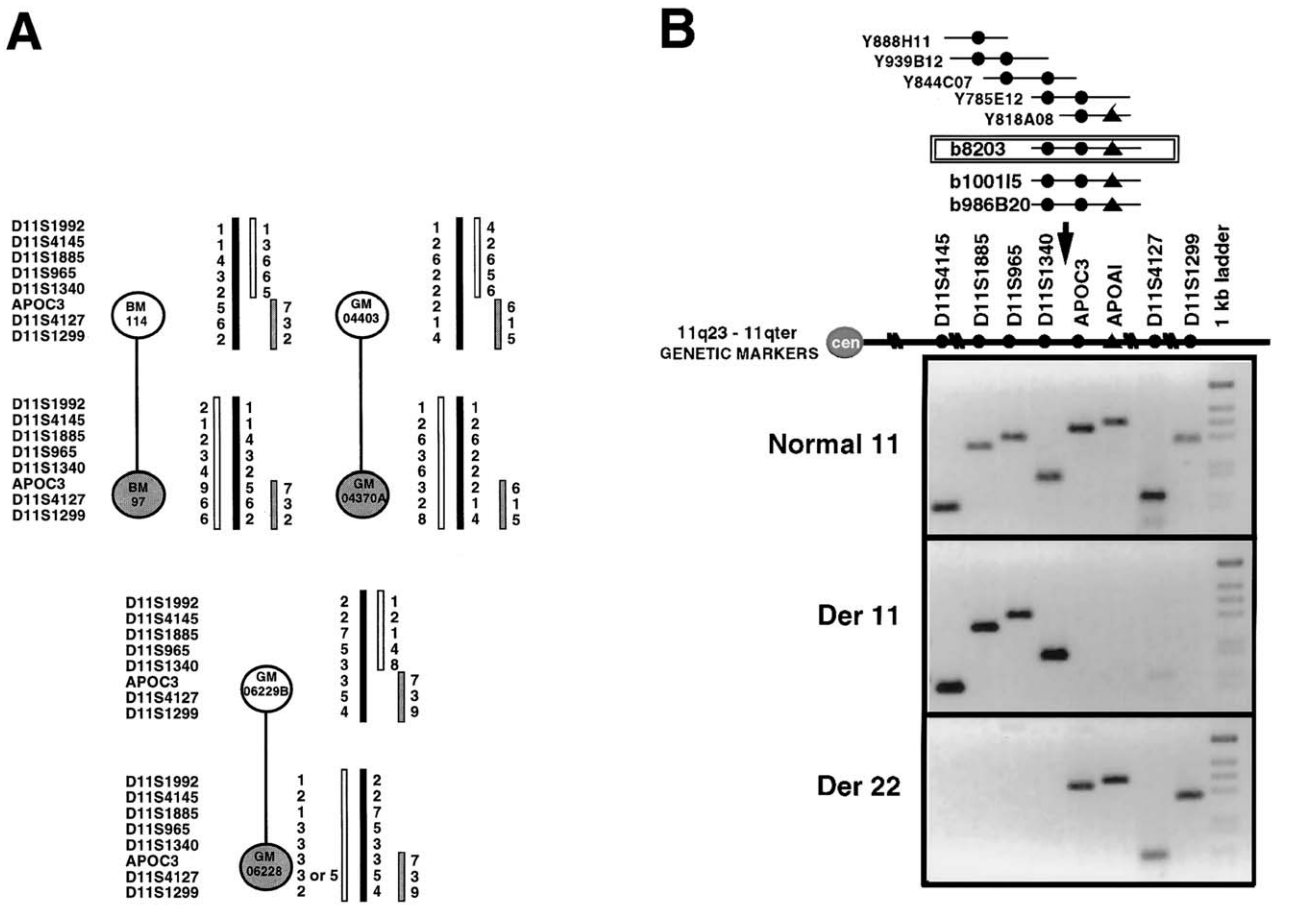

Figure 1 Haplotype analysis of t(11;22) carriers and offspring with der(22). *A,* Genetic markers that span the 11q23 region are ordered from the top (most centromeric) marker, D11S1992, to the bottom (most telomeric) marker, D11S1299. Two cases—BM97 and GM04370A—were informative for trisomy at the APOC3-tetra polymorphic locus. Haplotypes for carriers BM114 and GM06229B were determined from somatic-hybrid cell lines. *B,* PCR analysis of hamster-human somatic-hybrid cell lines generated from carriers BM114 and GM06229B. In both carriers (BM114 is shown), the breakpoint occurred between D11S1340 and APOC3-tetra, as is indicated by the *arrow*. Above the PCR analysis is the existing YAC contig on 11q23 (Tunnacliffe et al. 1999) and three BAC clones—b8203, b1001I5, and b986B20—that were positive with markers D11S1340 and APOC3-tetra. The BAC clone, b8203 (*boxed*), was used in FISH analysis.

the clones. Once the integrity of the clones was verified, PCR was performed on 100 ng template DNA, as described above.

FISH

The BAC clone 8203 was isolated from the RPCI-13 human BAC library (BACPAC Resource Center, Department of Cancer Genetics, Roswell Park Cancer Institute) and was used for FISH mapping studies. Preparation of probes and hybridization of samples for FISH studies were performed as described elsewhere (Shaffer et al. 1994). The BAC clone 8203 was labeled with digoxigenin 11-dUTP (Boehringer Mannheim), and the chromosome 22–telomere probe (Ning et al. 1996) was labeled with biotin 14-dATP (Boehringer Mannheim), by means of the nick-translation method (BioNick Labeling System; Gibco BRL). The chromosome 11–centromere probe was obtained as labeled with biotin (Vysis).

Results

Haplotype Analysis of Patients with der(22)

To identify the region on 11q23 that harbors the t(11;22) breakpoint, haplotype analysis was performed, with the use of ordered genetic markers that were previously integrated into a YAC-based physical map of 11q23 to 11qter (Tunnacliffe et al. 1999), on five unrelated patients (BM97, BM317, GM03371, GM04370A, and GM06228) with der(22) syndrome. Because individuals with der(22) have an extra chromosome, 11q23-qter, a haplotype is informative when three alleles are present, indicating trisomy at that

locus. All five offspring with der(22) exhibited one or two distinct alleles for the markers D11S1992–D11S1340 (fig. 1*A*). For the next distal marker, APOC3-tetra, three different alleles were present in the der(22) offspring BM97 and GM04370A. The other patients were uninformative (data not shown). For BM97, alleles 9, 5, and 7 were detected; for GM04370A, alleles 3, 2, and 6 were detected (fig. 1*A*). Therefore, this locus was trisomic in both patients with der(22). The haplotype results suggested that the breakpoint on 11q23 occurred between D11S1340 and APOC3-tetra. The APOC3-tetra polymorphic marker is located within the *Apolipoprotein C3* gene. *APOC3* comprises a small gene-family cluster that includes *APOA4* and *APOA1* on 11q23 (fig. 1*A*) (Karathanasis 1985; Mietus-Snyder et al. 1990).

It is of interest to note that five carriers and their offspring with der(22) were mother-daughter pairs, and one was a father-son pair (BM85, a patient with der(22) syndrome; Funke et al. 1999). This apparent sex bias is consistent with previously reported data indicating that the carrier parent of offspring with der(22) is female in the majority of cases (Fraccaro et al. 1980; Zackai and Emanuel 1980).

Somatic-Hybrid Analysis

To confirm the results obtained by haplotype analysis and to extend our studies of the breakpoint region, we generated hamster-human somatic-hybrid cell lines from the t(11;22) carriers BM114 and GM06229B (fig. 1*B*). In the somatic-hybrid cell lines, the normal and derivative chromosomes are physically separated from each other in distinct cell lines and can therefore be analyzed by use of monomorphic PCR-based markers. To verify the integrity of the 11q23 region in the derivative and normal chromosomes in the hybrid cell lines, we genotyped them with the genetic markers shown in figure 1*A*. A subset of the 11q23 genetic markers was present on either the der(11) or der(22) cell lines (fig. 1*B*). The markers that were distal (telomeric) to D11S1340 were absent from the der(11) chromosome, and the markers that were proximal (centromeric) to D11S1340 were absent from the der(22) chromosome. On the basis of these results, the t(11;22) breakpoint in both carriers occurred between the genetic markers D11S1340 and APOC3-tetra (fig. 1*B*), as is suggested by the haplotype analysis.

FISH Mapping of Carriers and Patients with der(22) Syndrome

The aforementioned PCR studies of the somatic-hybrid cell lines demonstrated that the breakpoint occurred between two genetic markers in the two carriers. To determine whether the breakpoints were similar in additional carriers, we performed FISH mapping studies of seven unrelated carriers, including BM114 and GM06229B. The YAC-contig physical map of 11q23 was examined (Tunnacliffe et al. 1999) to generate probes to screen the BAC library for FISH probes. We generated a monomorphic gene-based PCR marker that is amplified from the *APOA1* gene, which is tightly linked to the *APOC3* gene on 11q23 (Meitus-Snyder et al. 1990). We obtained 10 BAC clones, three of which were positive for the genetic markers APOC3-tetra and D11S1340 and thus spanned the breakpoint in the two carriers, BM 114 and GM 06229B (fig. 1*B*). Because the two markers were present within a single BAC clone, the distance between them could not be greater than the size of the human genomic-DNA insert of the BAC, which, on average, is 166 kb (BACPAC Resource Center, Department of Cancer Genetics, Roswell Park Cancer Institute). We chose BAC clone 82O3 (fig. 1*B*) for use in FISH studies performed on fibroblast or lymphoblast cell lines derived from the carriers and patients with der(22) syndrome. In all seven of the carrier cell lines, the BAC clone hybridized to the normal chromosome 11 and to both the der(11) and der(22) chromosomes, indicating that the clone crossed the t(11;22) breakpoint (fig. 2). For the der(22) cell lines, positive hybridization was detected on both normal chromosomes 11 and the der(22) chromosome.

Sequence Analysis

To further delineate the breakpoints in the somatic-hybrid cell lines, we examined the genomic sequence of BAC clone b1030 (GenBank accession number AC007707), which covered the region of interest between D11S1340 and APOC3. We used a PCR-based approach to systematically divide and narrow the breakpoint region. For this approach, eight PCR markers (b1030-1–b1030-8) were created from the genomic sequence, in progressively smaller intervals, and were used to examine the DNA from the somatic-hybrid lines of BM114 and GM06229B (fig. 3*A*). We determined that the *ZNF259* gene (GenBank accession number NM003904), for which the precise genomic location had not been previously reported, mapped within this interval. The *ZNF259* gene encodes a putative a Zn-finger protein that is homologous to the murine *Zpr1* gene (Galcheva-Gargova et al. 1996). In both carriers, the breakpoint occurred between the markers b1030-5, which is present on the der(11) chromosome, and b1030-6, which is present on the der(22) chromosome (fig. 3*A*). The t(11;22) breakpoint is 24 kb centromeric to the translation initiation site of the ZNF259 gene, suggesting that it does not disrupt the gene.

The breakpoint on 11q23, between b1030-5 and -6, was within a 190-bp interval in both carriers (fig. 3*B*). Within the 190-bp breakpoint interval in the sequenced

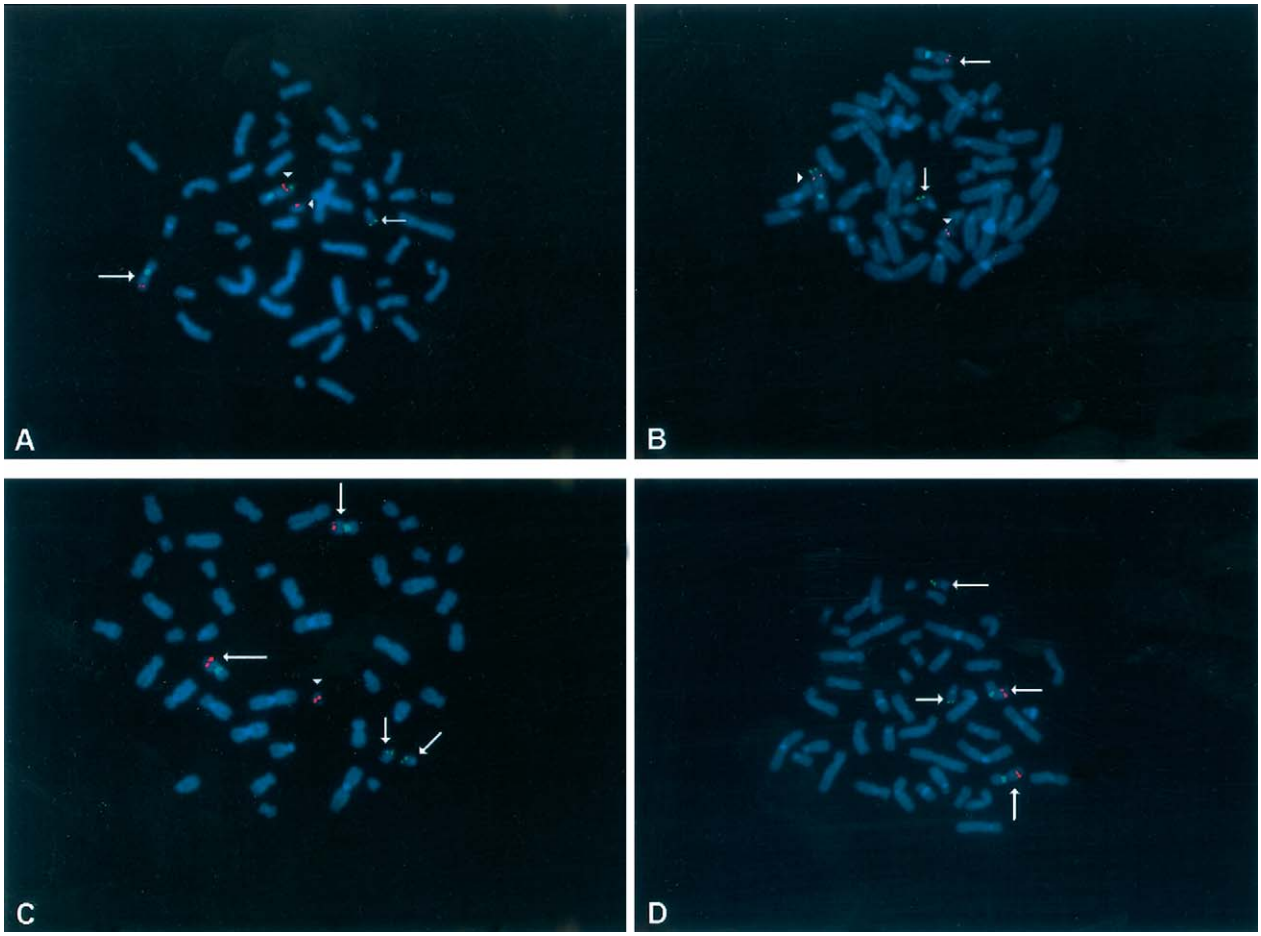

Figure 2 Results of FISH mapping studies on t(11;22) carriers and offspring with der(22). *A–D,* Metaphase chromosomes of t(11;22) carriers BM114 and GM04403, der(22) syndrome patient BM317, and a normal individual, respectively, were hybridized with a fluorescent probe (*red*) from BAC 8203. A chromosome 22–specific telomere probe (*green*) (Ning et al 1996) and a chromosome 11–specific centromere probe (*green*) (Vysis) were also used. Arrows indicate normal chromosomes; arrowheads indicate derivative chromosomes.

clone is a 108-bp complex AT repeat containing nine unequally spaced copies of TAAATAT that is not interrupted by C or G (fig. 3*B*). That the t(11;22) breakpoint occurs within the same precise interval suggests that the small AT-rich repeat may be prone to rearrangement.

Discussion

AT-Rich Repeat

It has been estimated that 1/2,000 individuals carries a de novo balanced translocation and is at risk of having unbalanced offspring (Warburton 1991). The frequency of translocations in couples with recurrent spontaneous abortions is 20-fold higher than that in the general population (Michels et al. 1982; Campana et al. 1986). To understand the molecular basis of reciprocal translocations, we examined the t(11;22) breakpoint interval on 11q23. This is because the t(11;22) constitutional translocation is the most common non-Robertsonian translocation in humans (Fraccaro et al. 1980; Zackai and Emanuel 1980). In the present study, we found that, in several unrelated carriers, the t(11;22) breakpoint interval mapped to a single BAC clone. We were then able to narrow the breakpoint interval on 11q23, in the derivative chromosomes from two unrelated carriers, to a 190-bp region that contains an AT-rich repeat.

Dinucleotide-rich repeats, such as chemical-induced fragile sites, are prone to rearrangements. Folate-sensitive fragile sites consisting of CG-rich trinucleotide repeats may be involved with rearrangements (Sutherland and Richards 1995, 1999). A p(CCG)*n* fragile site, termed "FRA11B," is present in the 11q23 region (Jones et al. 1995). The fragile site is in the general vicinity of an 11q23-24 deletion disorder termed Jacobsen syndrome (Michaelis et al. 1998; Tunnacliffe et al. 1999). Since this region also harbors the t(11;22) translocation breakpoint, we examined the physical map of 11q23

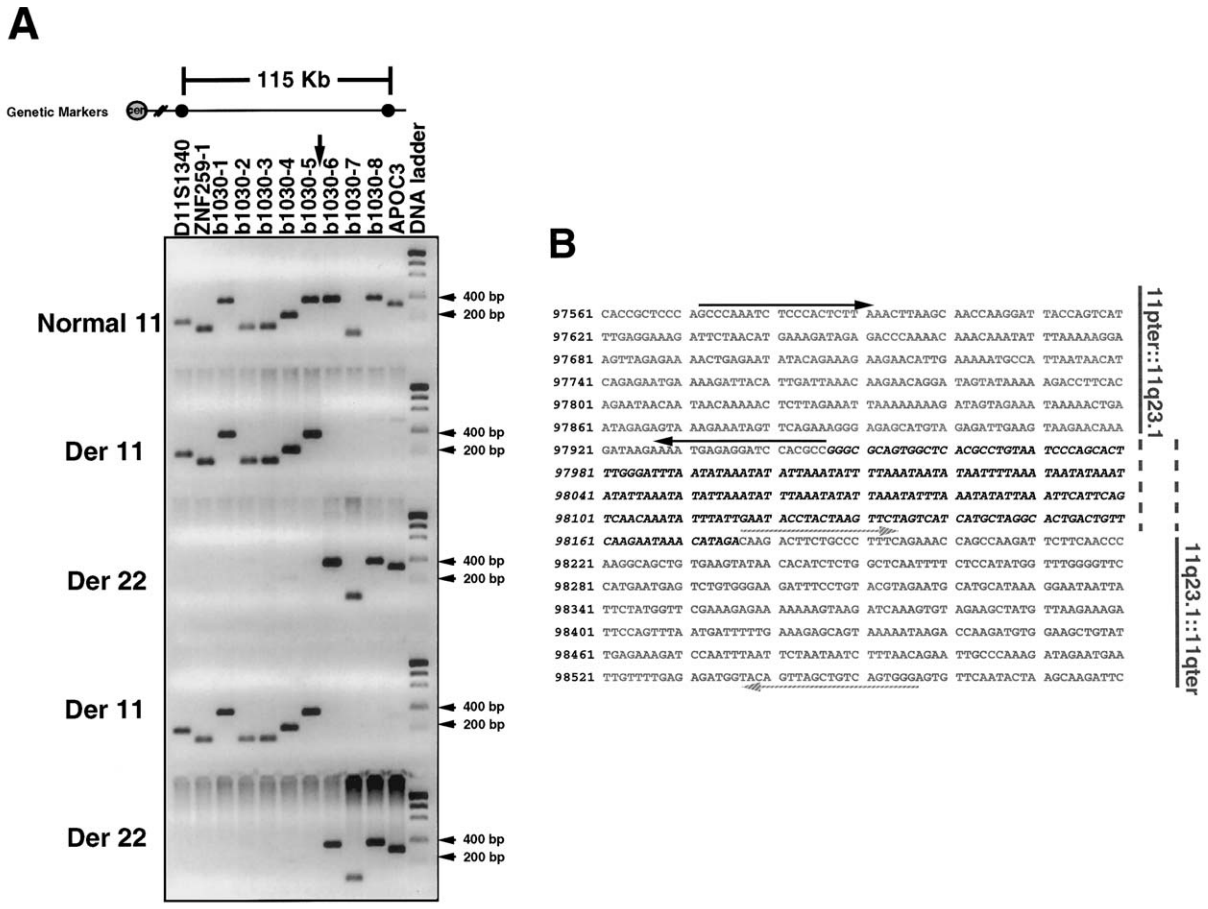

Figure 3 Definition of t(11;22) translocation breakpoint in hamster-human somatic-hybrid cell lines. *A,* PCR analysis of BM114 and GM06229B hybrid cell lines. The markers were generated from the available human genomic sequence of the insert of BAC clone b1030 (AC007707), shown oriented from centromere to telomere (left to right), and span a distance of 115 kb, as indicated. The nucleotide positions of markers created from insert sequence of clone b1030 are indicated in parentheses: D11S1340 (2011–2155), ZNF259-1 (64509–64553), b1030-1 (77917–78289), b1030-2 (85636–85783), b1030-3 (91969–92118), b1030-4 (96538–96759), b1030-5 (97572–97949), b1030-6 (98177–98566), b1030-7 (102824–102928), b1030-8 (104820–105208), and APOC3-tetra (116789–117113). The arrow indicates the position of the t(11;22) breakpoint. Shown from top to bottom are the PCR results of BM114 normal chromosome 11, BM 114 der(11) chromosome, BM 114 der(22) chromosome, GM06229B der(11) chromosome, and GM06229B der(22) chromosome. *B,* Sequence from b1030 at the junction of the der(11) and der (22)chromosomes of BM114 and GM06229B. Blackened arrows indicate the positions of the forward and reverse primers of the b1030-5 PCR product; dotted arrows indicate positions of the forward and reverse primers of the b1030-6 PCR product. Sequences at the breakpoint region are highlighted in bold.

(Tunnacliffe et al. 1999). FRA11B was located in the genomic interval that encompasses the *CBL2* proto-oncogene (Jones et al. 1995). *CBL2* is 2–3 Mb telomeric to *APOC3* (Tunnacliffe et al. 1999) and, therefore, the t(11;22) breakpoint (fig. 1*B*). On the basis of these findings, we conclude that the t(11;22) breakpoint does not occur near FRA11B.

It is possible that the 190-bp AT-rich repeat constitutes a minisatellite or variable number of tandem repeat (VNTR). Minisatellites containing complex AT- or CG-rich repeats constitute unstable regions of the genome. These elements are polymorphic both in sequence within each repeat and in repeat number (Jeffreys et al. 1990). These regions may represent some of the most unstable loci in the human genome. A distamycin A–sensitive fragile site, FRA16B, on chromosome 16 is composed of an irregular AT-rich repeat in normal individuals (Schmid et al. 1986). It is prone to breakage by AT-rich repeat–specific antibiotics when expanded (Yu et al. 1997). These results suggest that AT-rich repeats constitute chemical-induced fragile sites and that they may be involved in mediating chromosome instability. It is not known whether the AT-rich repeat at the breakpoint is polymorphic in humans. Nevertheless, nonhypervariable minisatellites have been shown to stimulate in vitro homologous recombination events and may therefore represent unstable regions in the genome (Boan et al. 1998).

Alternatively, the AT-rich repeat itself may not be the sole element in the region that confers susceptibility to rearrangements. It is possible that the t(11;22) breakpoint on 11q23 could be mediated, in part, by flanking sequences. This has been shown to be the case for three different CG-rich minisatellites—MS31A, MS32, and MS205—that are prone to rearrangements (Murray et al. 1999). A recombination hot spot that is immediately adjacent to, yet outside of, the repeat can confer chromosomal rearrangements (Murray et al. 1999). Unfortunately, there were no common sequences or motifs that flank these repeats; therefore, the sequence alone may not be sufficient to define a hot spot (Jeffreys et al. 1998; Murray et al. 1999). We examined the interval that flanks the AT-rich repeat for sequence motifs of interest. A 635-bp complex repeat, which consists of 56 copies of GAATATATATAT, with some variation, and which is 5,600 bp distal to the 108-bp AT-rich repeat (nucleotides 103677–104312; GenBank accession number AC007707), was identified. It is possible that this repeat may play a role in the generation of instability in the region on 11q23. The mechanism for this repeat acting at a distance is unknown.

Interestingly, AT-rich repeats are present in multiple copies within each LCR22. Examination of the sequence of PAC699J1 (GenBank accession number AC008103), a clone that maps to the proximal 3-Mb LCR22 (Edelmann et al. 1999*a*), revealed a series of AT-rich repeats (nucleotides 44752–50557). One repeat spans a region from nucleotides 45611–46761, for a distance of 1,150 bp. Another AT-rich repeat spans the region from nucleotides 47621–48213, for a distance of 592 bp, and a third spans the region from nucleotides 49084–50558, for a distance of 1,478 bp.

An AT-rich motif has been implicated in the novel, reciprocal t(17;22)(q11;q11) translocation in a family with neurofibromatosis type 1 (NF1) (Kehrer-Sawatski et al. 1997). The exchange event occurred between an AT-rich repeat within intron 31 of the *NF1* gene on chromosome 17 and a similar repeat on 22q11 (Kehrer-Sawatski et al. 1997). The repeated sequences map to the LCR22s (Edelmann et al. 1999*b*). That AT-rich repeats are involved in two distinct reciprocal translocations, the novel t(17;22) (Kehrer-Sawatski et al. 1997) and the recurrent t(11;22) described here, suggests that this repeat motif may be prone to recombination events leading to rearrangements. That the LCR22s also have AT-rich repeats could implicate them in the meiotic homologous recombination events that lead to 22q11 deletions and duplications that are associated with congenital anomaly disorders. Further studies must be performed to determine whether this hypothesis is correct.

LOH on 11q23

A high incidence of loss of heterozygosity (LOH) of 11q23-qter has been seen in a variety of solid tumors. Many breakpoints were centered around the *APOC3* gene, near the t(11;22) breakpoint. In breast cancer, one of the regions that showed LOH on 11q23 surrounded the *APOC3* gene (Laake et al. 1997). However, the breakpoints varied among tumors and extended over large distances in either direction (Laake et al. 1997). The *APOC3* gene and the surrounding region showed LOH in oral cancer (Uzawa et al. 1996), ovarian cancer (Launonen et al. 1998), and lung cancer (Wang et al. 1999), among others. This LOH extended from the gene for a significant distance in either direction, indicating that the breakpoints in these cancers were variable in length. On the basis of these findings, it is unlikely that the AT-rich repeat mediated the majority of large rearrangements in malignant tumors.

Conclusion

The recurrent t(11;22) breakpoint on both 11q23 and 22q11 occurs in regions that are prone to rearrangements. The breakpoint on 22q11 occurs in one of nine LCR22s that span the 22q11 region (Edelmann et al. 1999*b*), suggesting that the configuration of this LCR22 may be more favorable to the t(11;22) translocation. In this report, we show that the 11q23 breakpoint occurs in an AT-rich repeat. That the LCR22s contain AT-rich repeats suggests that the AT-rich repeats may mediate the rearrangements on chromosomes 11 and 22. To determine the precise molecular mechanism that causes this recurrent translocation, it will be necessary to clone and sequence the t(11;22) breakpoint junction. Efforts are currently under way to identify the breakpoint junction.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Coriell Cell Repositories, http://locus.umdnj.edu/ccr/ (for cell lines purchased from the NIGMS cell repository)
GenBank, http://www.ncbi.nlm.nih.gov/Genbank/
Online Mendelian Inheritance in Man (OMIM), http://www

.ncbi.nlm.nih.gov/Omim/ (for VCFS [192430], DGS [188400], and CES [115470])

BACPAC Resource Center, Department of Cancer Genetics, Roswell Park Cancer Institute, http://bacpac.med.buffalo.edu/ (for high-density gridded membranes containing the 21.8X, RPCI-13 BAC library)

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