© 1999 Oxford University Press
Human Molecular Genetics, 1999, Vol. 8, No. 7 **1157–1167**

ARTICLE

A common molecular basis for rearrangement disorders on chromosome 22q11

Lisa Edelmann, Raj K. Pandita, Elizabeth Spiteri, Birgit Funke, Rosalie Goldberg, Nallasivam Palanisamy[1], R. S. K. Chaganti[1], Ellen Magenis[2], Robert J. Shprintzen[3] and Bernice E. Morrow[+]

Department of Molecular Genetics, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, USA, [1]Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, Box 391, New York City, NY 10021, USA, [2]Child Development Rehabilitation Center, Oregon Health Science University, 707 Southwest Gaines Road, Portland, OR 97201, USA and [3]Center for the Diagnosis, Treatment and Study of Velo-Cardio-Facial Syndrome, State University of New York Health Science Center, New York, NY 13210, USA

Received February 28, 1999; Revised and Accepted April 29, 1999

The chromosome 22q11 region is susceptible to rearrangements that are associated with congenital anomaly disorders and malignant tumors. Three congenital anomaly disorders, cat-eye syndrome, der(22) syndrome and velo-cardio-facial syndrome/DiGeorge syndrome (VCFS/DGS) are associated with tetrasomy, trisomy or monosomy, respectively, for part of chromosome 22q11. VCFS/DGS is the most common syndrome associated with 22q11 rearrangements. In order to determine whether there are particular regions on 22q11 that are prone to rearrangements, the deletion end-points in a large number of VCFS/DGS patients were defined by haplotype analysis. Most VCFS/DGS patients have a similar 3 Mb deletion, some have a nested distal deletion breakpoint resulting in a 1.5 Mb deletion and a few rare patients have unique deletions or translocations. The high prevalence of the disorder in the population and the fact that most cases occur sporadically suggest that sequences at or near the breakpoints confer susceptibility to chromosome rearrangements. To investigate this hypothesis, we developed hamster–human somatic hybrid cell lines from VCFS/DGS patients with all three classes of deletions and we now show that the breakpoints occur within similar low copy repeats, termed LCR22s. To support this idea further, we identified a family that carries an interstitial duplication of the same 3 Mb region that is deleted in VCFS/DGS patients. We present models to explain how the LCR22s can mediate different homologous recombination events, thereby generating a number of rearrangements that are associated with congenital anomaly disorders. We identified five additional copies of the LCR22 on 22q11 that may mediate other rearrangements leading to disease.

INTRODUCTION

Rearrangements of the q11 region of chromosome 22 are associated with multiple congenital anomaly disorders and malignant neoplastic disease. The most common rearrangement on 22q11 associated with malignancy is the balanced t(9;22) translocation in patients with acute lymphocytic leukemia (ALL; MIM 159555) and chronic myeloid leukemia (CML; MIM 151410). A characteristic Philadelphia chromosome t(9;22)(q34;q11) is present in these tumor cells (1). The balanced t(8;22) translocation is associated with Burkitt's lymphoma (BL; MIM 113970). The t(8;22) translocation disrupts the immunoglobulin light chain locus on 22q11 (2,3). Other rearrangements associated with malignant tumors include the t(11;22) translocation of Ewing sarcoma (MIM 133450) and the deletions or translocations of malignant rhabdoid tumors (MIM 601607) and meningiomas (MIM 156100).

Germline rearrangements on 22q11 occur in association with multiple congenital anomaly disorders. Cat-eye syndrome (CES; MIM 115470) (4), der(22) syndrome (5), velo-cardio-facial syndrome (VCFS; MIM 192430) (6) and DiGeorge syndrome (DGS; MIM 188400) (7) are all associated with 22q11 rearrangements. Because VCFS and DGS are caused by the same 22q11 deletion, they are often referred to as VCFS/DGS. In contrast to somatic rearrangements, which occur at distinct chromosomal sites, all three congenital anomaly disorders share a physical region of overlap on 22q11 (8–10). CES patients have a supernumerary bisatellited marker chromosome 22pter–q11 resulting from an inverted duplication of the proximal 22q11 region (4,11,12). Two distinct duplication breakpoints on 22q11 occur in CES patients

[+]To whom correspondence should be addressed. Tel: +1 718 430 4273; Fax: +1 718 430 8778; Email: morrow@aecom.yu.edu

Downloaded from https://academic.oup.com/hmg/article/8/7/1157/609436 by guest on 21 August 2022

(13). The proximal and distal duplication breakpoints border the region deleted in VCFS/DGS (13). The constitutional t(11;22) translocation is the most common recurrent non-Robertsonian translocation in humans (5,14). Normal balanced t(11;22) translocation carriers are at risk of giving birth to offspring with der(22) syndrome, resulting from a 3:1 meiotic non-disjunction (5,14). The region on 22q11 that is trisomic overlaps with the interval that is duplicated in some CES patients and deleted in most VCFS/DGS patients (10,15).

In contrast to der(22) syndrome and CES, which are rare disorders, VCFS/DGS occurs more often in the general population, occurring with an estimated frequency of 1/4000 live births (16). It is the most common microdeletion disorder in humans. The main clinical findings of VCFS/DGS include cleft palate, characteristic facies, conotruncal heart defects, aplasia or hypoplasia of the thymus gland, hypocalcemia and learning disabilities, and behavioral and psychiatric disorders (6,7,17). Most cases are sporadic, suggesting that the 22q11 region is prone to deletions. To determine the molecular basis of the deletions on 22q11, a physical map was developed and haplotype analysis was performed on 105 VCFS/DGS patients using ordered genetic markers (18). Three classes of deletion were identified in the patients. The most common deletion is 3 Mb in size and occurs in 90% of the patients with deletions. A 1.5 Mb deletion occurs in 7% of the patients with 22q11 deletions, and unique deletions or translocations have been found in a few rare patients (18–24). To define the intervals spanning the chromosome breakpoints that result in the 3 Mb deletion, high-resolution physical maps were developed (25). Because low copy repeats span the 22q11 region, it has been hypothesized that they may be involved in chromosome rearrangements (26,27). Haplotype analysis revealed that the common 3 Mb deletion occurred in the vicinity of a low copy repeat, denoted VCFS-REP (25). The common 3 Mb deletion is mediated by both meiotic inter- and intra-chromosomal homologous recombination events between the two VCFS-REPs (25,28).

In this report, we show that all three classes of rearrangement in VCFS/DGS patients occur within similar low copy repeats. We renamed the repeats, low copy repeat on 22q11, or LCR22s, because they also mediate rearrangements for CES and der(22) syndrome. In addition, we describe one family with an interstitial duplication of the same 3 Mb region that is deleted in VCFS/DGS patients. We present models to explain how the LCR22s could mediate distinct chromosome rearrangements. Finally, we characterized five additional LCR22s in the 22q11 region, and we propose that they may mediate chromosome rearrangements leading to other disorders.

RESULTS

Mapping of chromosome 22q11 breakpoints in VCFS/DGS patients

To determine the molecular basis of 22q11 rearrangements, a physical mapping approach was undertaken. A key component of this approach is the availability of a high-resolution physical map of the 22q11 region (25,29). A schematic representation of the physical map is depicted in Figure 1. A second critical component is a set of well-defined patients with 22q11 chromosome rearrangements. To define the deletion end-points in the patients, haplotype analysis was performed with 16 ordered genetic markers (18,25). The patients were categorized according to the extent of the deletion or translocation. As described previously (18), most patients have a 3 Mb deletion, some have a nested 1.5 Mb deletion and a few rare patients have unique deletions or rearrangements.

To define precisely the interval containing the chromosome breakpoints for three classes of 22q11 rearrangements, two copies of chromosome 22, one deleted or rearranged and the other normal, were separated in hamster–human somatic hybrid cell lines generated from different patients. The deleted copy of chromosome 22q11 from three unrelated VCFS/DGS patients with the common 3 Mb deletion, BM41, BM293 and BM308, was analyzed by PCR with a set of ordered, high density, PCR-based, markers that span the region of interest on 22q11. The PCR markers were developed as part of the physical mapping process of chromosome 22q11 (18,25). The advantage of this method is that it allows for the precise definition of the breakpoint region, which is possible because the average spacing of PCR markers in the vicinity of the chromosome breakpoints is 8.5 kb (25). Therefore, the regions containing the chromosome breakpoints will be defined at a resolution of 8.5 kb. Representative results for BM41 are shown in Figure 2A. We found that the 3 Mb deletion occurred between the PCR marker 92D11T7 and the restriction fragment-based marker, BM 8.1 (*Bam*HI, 4.5 kb) (Figs 1 and 2A). Both markers lie ~8.5 kb from the low copy repeat markers (Fig. 2A). Based on this analysis, we show that the 3 Mb deletion occurs within the 3 Mb LCR22s (arrows A and D in Fig. 2A).

A similar strategy was undertaken to define precisely the proximal and distal breakpoints for patients with the 1.5 Mb deletion. For this study, we developed somatic hybrid cell lines from the VCFS/DGS patient BM15 (29) and examined them by PCR (Fig. 2B). The 1.5 Mb deletion in BM15 occurred between the PCR markers 92D11T7 and ZNF74 (Fig. 2B). The proximal breakpoint was identical to the proximal breakpoint in the patients with the 3 Mb deletion (Fig. 2A and B). The 1.5 Mb distal breakpoint occurred between the markers 444P24SP6 and ZNF74 (Figs 1 and 2B). The distal breakpoint occurred in the same vicinity as the constitutional t(11;22) breakpoint and the distal deletion breakpoint of the VCFS/DGS patient BM8, who has a unique nested proximal deletion breakpoint (15). When taken together, these results suggest that this interval is prone to rearrangements. The proximal and distal breakpoints in BM15 occurred in intervals containing low copy repeats or LCR22s (Fig. 2B). The average resolution of PCR markers in the distal 1.5 Mb breakpoint interval is 15 kb (15). Markers 444P24SP6 and ZNF74 are ~15 kb from the nearest low copy repeat marker (15). A gap is present in the physical map within the central portion of the LCR22 in the 1.5 Mb distal deletion breakpoint interval (Fig. 1). The gap is present because the bacterial clones that are anchored to the interval do not span the LCR22. Furthermore, the BAC and PAC libraries (30) were underrepresented in this region. Nevertheless, the ends of all the clones that extend into the gap contain markers that comprise the LCR22s. The clones that will fill the gap will most likely contain repetitive sequences. Due to the repetitive nature of the markers in this interval and the number of LCR22s that map to 22q11, it has not yet been possible to close this gap.

Several years ago, a VCFS/DGS patient called ADU was reported to carry a balanced t(2;22) translocation that disrupted the 22q11 region (19). To define the ADU breakpoint further, we developed somatic hybrid cell lines from patient ADU (Fig. 2A). The ADU breakpoint separates the proximal breakpoint from the distal breakpoint in 3 Mb deleted patients into two derivatives,

Downloaded from https://academic.oup.com/hmg/article/8/7/1157/609436 by guest on 21 August 2022

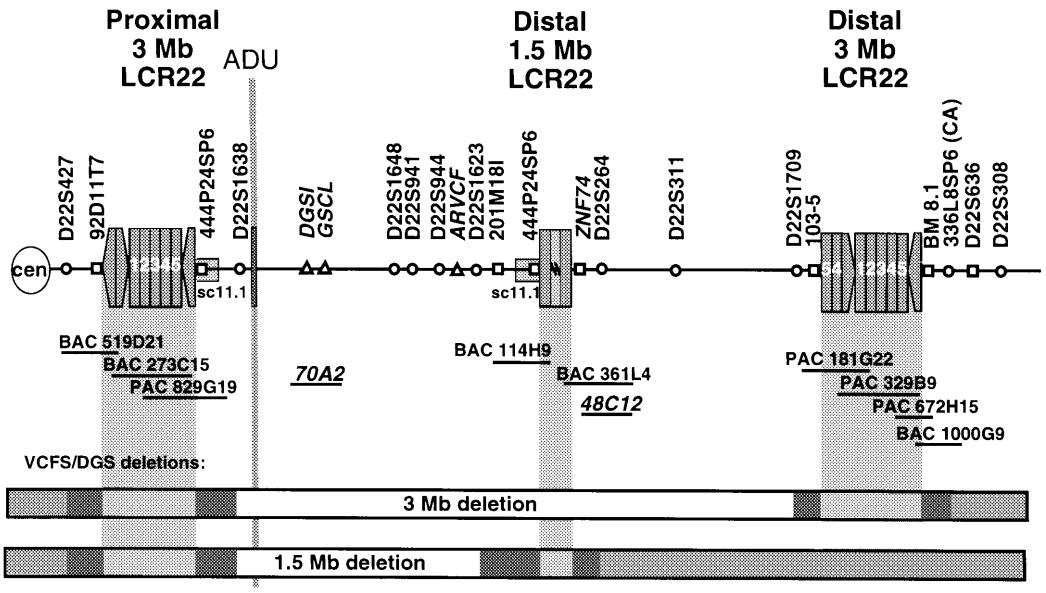

Figure 1. Schematic representation of the physical map of the 22q11 region associated with VCFS/DGS. The ordered PCR-based markers are indicated above the line representing chromosome 22q11. Details of the physical map are described (25). Polymorphic markers are denoted by circles, monomorphic markers by squares and gene-based markers by triangles. The three LCR22s are indicated as shaded clusters. The proximal and distal 3 Mb LCR22s contain a set of genes or pseudogenes that are numbered [1 = *GGT-Rel*, 2 = *GGT*, 3 = *V7-Rel*, 4 = *POM121L* and 5 = *BCRL* (25)]. Inverted sub-repeats consisting of anonymous genomic markers are indicated as inverted triangles in each LCR22 (25). The physical map of the distal 1.5 Mb LCR22 is not yet complete as indicated by the hatched lines (15). The clones that constitute the minimal tiling path across each LCR22 as well as the cosmids 70A2 and 48C12, used for FISH mapping, are shown below the line. The bars underneath the physical map indicate the positions of the 3 and 1.5 Mb VCFS/DGS deletions. The positions of the breakpoints as determined by haplotype analysis are indicated as dark shaded regions in the bars (25). The region containing the 1.5 and 3 Mb chromosome breakpoints as determined by somatic hybrid analysis is indicated as a lightly shaded interval that extends from the LCR22 clusters to the bars. The positions of the ADU breakpoint and the mini-LCR22 are shown.

der(2) and der(22). PCR markers that are centromeric to the t(2;22) breakpoint are absent in the der(2) chromosome, and markers distal to the breakpoint are missing in the der(22) chromosome. Markers 1152H5T7–39G5T7 are present on both derivatives and map to the LCR22s (Fig. 2A). The ADU breakpoint has been cloned and sequenced (Fig. 1) (31,32). The breakpoint on 22q11 maps within the sequenced fosmid clone, 41C7 (GenBank accession no. AC000095; B. Roe, University of Oklahoma, OK). We examined the region that surrounds the site of the ADU breakpoint on 22q11 and identified sequences that comprise the LCR22s. One of the genes or pseudogenes that map to each LCR22, termed *POM121L*, lies 10 kb centromeric to the ADU breakpoint. Therefore, the balanced translocation breakpoint of ADU occurs in the vicinity of a mini-LCR22, containing a small subset of the repeated markers.

Another type of low copy repeat is present in the 22q11 region; it is referred to as the *sc11.1* locus (26,27). Two *sc11.1* loci map to the 22q11 region as determined by interphase fluorescence *in situ* hybridization (FISH) mapping studies (27). The positions of the two *sc11.1* loci are indicated in Figure 1. One maps just distal to the proximal LCR22 and the second maps just proximal to the distal 1.5 Mb LCR22 (Fig. 1). Both are deleted in all patients with the 1.5 and 3 Mb VCFS deletion by interphase FISH mapping studies (20), as well as by the PCR analysis presented in Figure 2.

Interstitial duplication of the 3 Mb region on 22q11

Both intra- and inter-chromosomal homologous recombination events mediate the 3 Mb deletion in VCFS/DGS patients (25,28). Inter-chromosomal recombination events between homologous chromosomes could theoretically mediate a duplication of the 3 Mb interval as well. We recently identified a patient, designated BM495, with a partial interstitial duplication of 22q11 as determined by FISH mapping studies (N25 probe, Oncor; data not shown). The patient, mother and grandmother are shown in Figure 3A. The patient is a 4-year-old girl who was diagnosed with developmental delay but found not to have either VCFS or CES. During infancy, the clinical findings of the patient were a failure to thrive, with marked hypotonia, sleep apnea and seizure-like episodes. More recent diagnoses include a delay of gross motor development, with poor fine motor skills, velopharyngeal insufficiency and a significant delay in language skills. In addition, her facial features appear mildly dysmorphic, with a narrow face and downslanting palpebral fissures. Her hearing and vision are normal, and she has no detectable cardiac anomalies.

Haplotype analysis was performed on three generations of this patient's family to determine the extent and mechanism of the duplication, as shown in Figure 3B. The normal mother, BM496, and grandmother, BM498, were found to carry the duplication. Both individuals have a history of pre-auricular ear pits; however, there is no evidence of ear pits or tags in BM495. Examination of the chromosomes in all three generations revealed a duplication that corresponds to the same 3 Mb region that is deleted in VCFS/DGS patients. The presence of three different alleles for the genetic markers encompassed in the region that is duplicated is consistent with a meiotic inter-chromosomal recombination event in an ancestor of BM498. To our knowledge, this is the first reported case of an interstitial duplication of the 3 Mb region in 22q11, excluding other parts of chromosome 22.

Downloaded from https://academic.oup.com/hmg/article/8/7/1157/609436 by guest on 21 August 2022

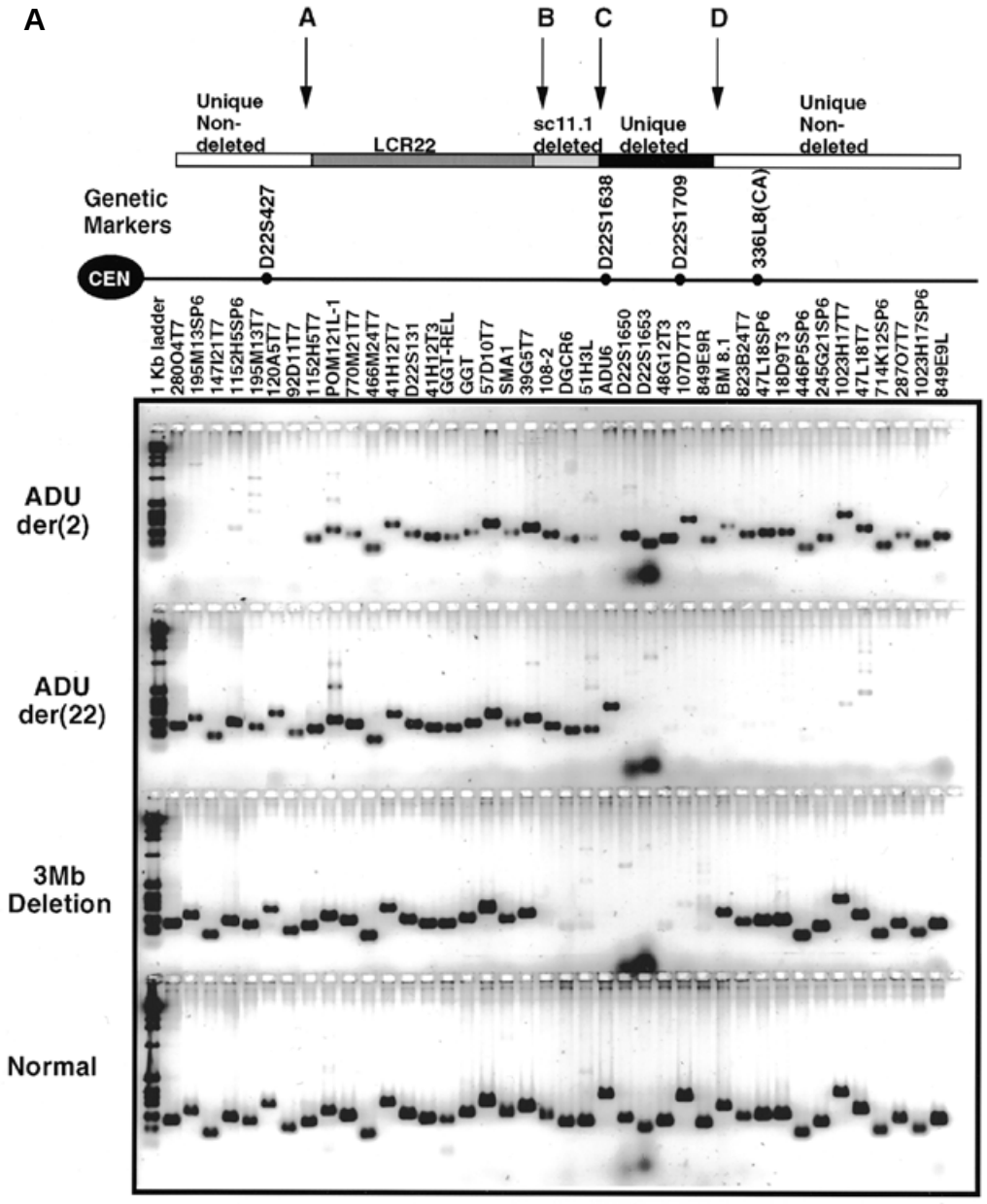

Figure 2. PCR analysis of chromosome 22q11 in hamster–human somatic hybrid cell lines from VCFS/DGS patients. (**A**) PCR analysis of the separated, der(2) and der(22) chromosomes of patient ADU and the 3 Mb deleted and normal chromosomes from BM41 is shown. PCR markers are listed below the line representing chromosome 22q11 (25). The position of the genetic markers with respect to the location of PCR markers is shown. The shaded bar below the arrows is a schematic description of the representative markers tested. Note that the bar is not drawn to scale. Arrow A demarcates the position of the proximal breakpoint of the 3 Mb deletion. The markers between the arrows A and B, 1152H5T7–39G5T7, are representative proximal and distal LCR22 markers. Representative markers from the *sc11.1* locus are between arrows B and C. Representative markers distal to arrow C are absent in the der(22) chromosome of patient ADU. Arrow D demarcates the position of the distal breakpoint of the 3 Mb deletion. (**B**) PCR analysis of the chromosome containing the 1.5 Mb deletion from patient BM15. The position of the genetic markers with respect to the location of PCR markers is shown. The shaded bar below the arrows is a schematic description of the representative markers tested. Note that the bar is not drawn to scale. Arrow A demarcates the position of the proximal breakpoint of the 1.5 Mb deletion. The markers between the arrows A and B are similar to those shown in (A). The *sc11.1* locus is between arrows B and C. Arrow D demarcates the distal breakpoint of the 1.5 Mb deletion.

To examine the mechanism of the duplication further, metaphase and interphase FISH mapping studies were performed with two cosmids, 70A2 and 48C12 (Figs 1 and 4). Probes derived from the cosmids were hybridized to metaphase and interphase chromosomes from a normal individual, patient BM495, her mother and grandmother. Because the pattern of hybridization of all three family members was identical, we show the results from BM495 exclusively. The order of the probes from the centromere in normal individuals is 70A2 followed by 48C12 (Figs 1 and 4A). The order of the probes from the centromere is 70A2–48C12–48C12–70A2 in BM495, 496 and 498 (Fig. 4B), demonstrating that the duplicated segment is inverted.

Downloaded from https://academic.oup.com/hmg/article/8/7/1157/609436 by guest on 21 August 2022

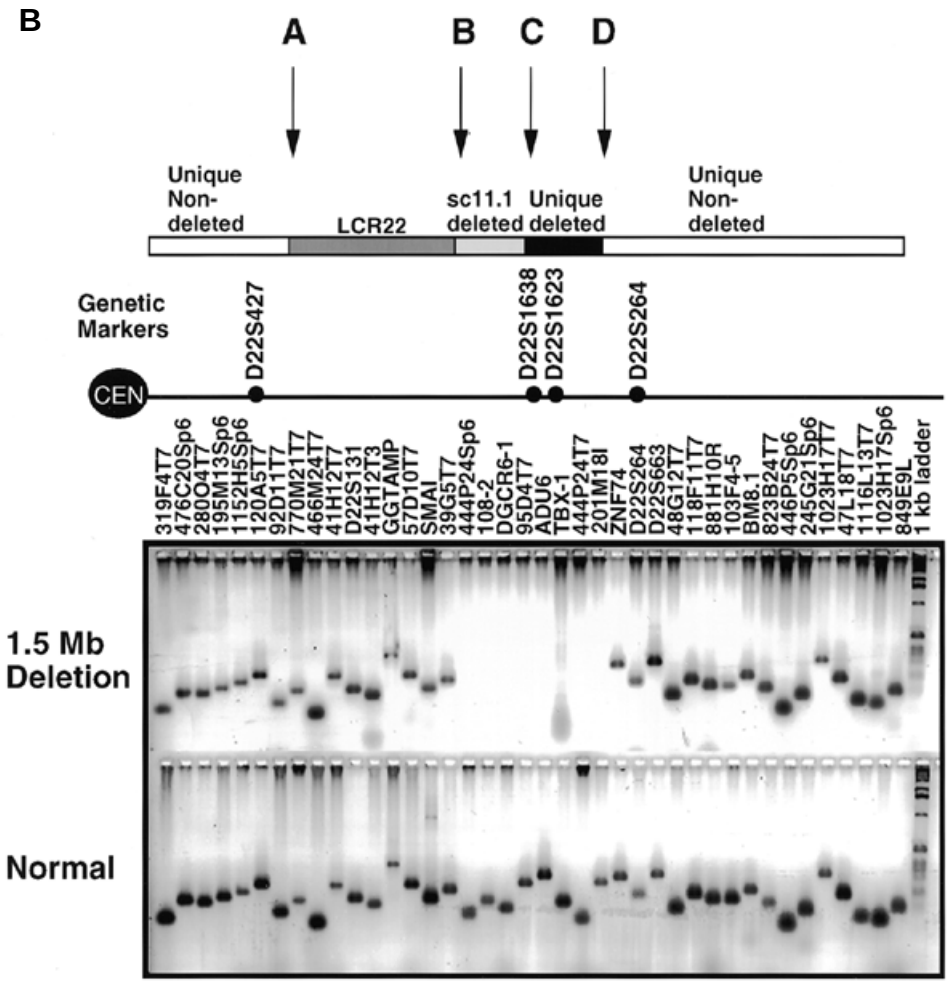

Figure 2. *Continued.*

Low copy repeats and rearrangements on 22q11

As described above, the 22q11 region is susceptible to rearrangements leading to VCFS/DGS. We found that all three classes of rearrangements associated with VCFS/DGS occur within LCR22s (Fig. 5). The duplication breakpoints for patient BM495 occur in the same proximal and distal LCR22s as for the VCFS/DGS patients with the 3 Mb common deletion (Fig. 5). The balanced t(11;22) translocation in normal carrier parents of der(22) syndrome patients occurs in the same LCR22 as for the distal 1.5 Mb deletion breakpoint in VCFS/DGS patients (Fig. 5) (15). Finally, the t(2;22) balanced translocation of patient ADU occurs within a mini-LCR22.

Two distinct CES chromosome duplication breakpoints have been mapped recently, a smaller type I and a larger type II breakpoint (13). The type I CES breakpoints occur between the genetic markers *D22S427* and *D22S1638* (13). Examination of the physical map shown in Figure 5 indicates that these are the same genetic markers that flank the proximal 3 Mb LCR22. The type II CES breakpoints occur in the interval flanked by *D22S1709* and *D22S636* (13) (Fig. 1). This is the same interval that contains the distal 3 Mb LCR22 (Fig. 5). In addition, some of the CES rearrangements are asymmetric; one type I and one type II breakpoint are present in individual patients (13). These data indicate that the proximal and distal 3 Mb LCR22s mediate a number of distinct rearrangements on 22q11.

To determine the basis of other rearrangements, we scanned the chromosome 22q11 region for additional copies of LCR22. The 3 Mb proximal and distal LCR22s contain a set of five genes or pseudogenes which include *GGT-Rel* (33), *GGT* (34), *V7-Rel* (35), *POM121L* (36) and *BCRL* (37,38). Two of the genes, *GGT* and *BCRL*, are repeated in clusters that span the 22q11 region (33,34,37,39,40), indicating that they could correspond to additional copies of the LCR22. To determine whether this is the case, we performed PCR analysis with markers from each of the five genes on YAC clones (40) and/or bacterial clones that were known to be positive for *GGT* and/or *BCRL* (Fig. 5). We identified five additional LCR22s that span the 22q11 region. Each contains a subset of genes and genomic markers that are characteristic for the LCR22s (Fig. 5).

A 1 Mb region within the immunoblobulin light chain locus that encompasses the LCR22 containing the *GGT.3* gene has been sequenced (41). This LCR22 is ~20 kb, less than one-tenth the size of the 3 Mb LCR22s. It contains markers corresponding to four of the five genes, all of which are pseudogenes (41); however, additional markers that comprise the 3 Mb LCR22s are missing, suggesting that there are structural

Downloaded from https://academic.oup.com/hmg/article/8/7/1157/609436 by guest on 21 August 2022

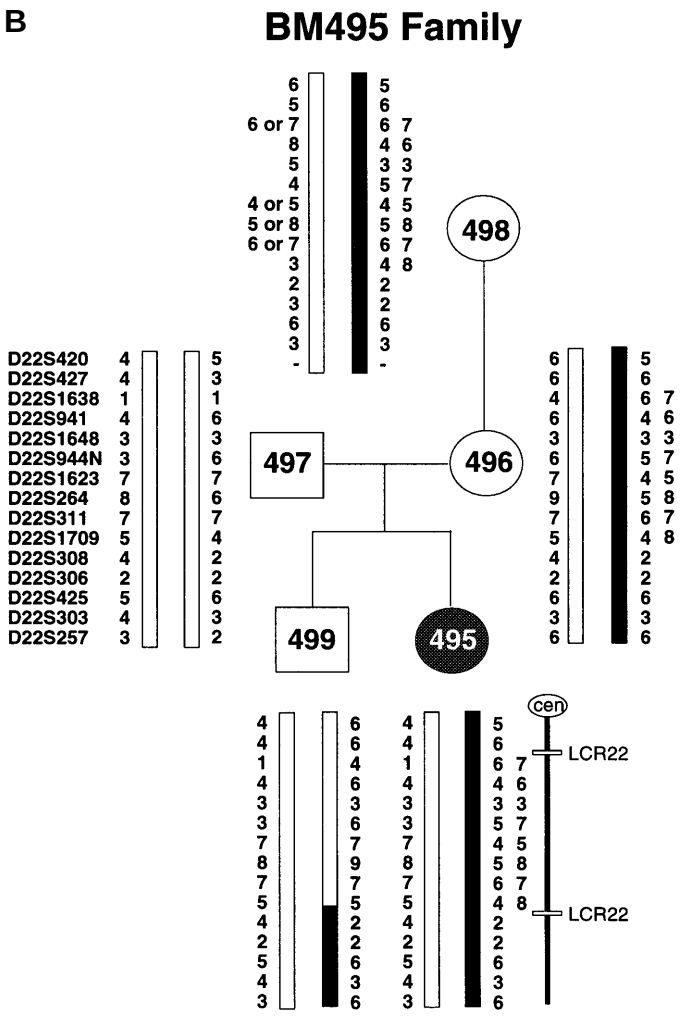

Figure 3. Photograph and haplotype analysis of BM495. (**A**) Photograph of BM498, 495 and 496, left to right. (**B**) Three generations were genotyped using a set of ordered genetic markers that span the 22q11 region as listed. The resulting haplotypes are illustrated. The origin of the duplicated chromosome is shown (black bar).

differences between the LCR22s that may play a role in the type of rearrangement that takes place on chromosome 22q11.

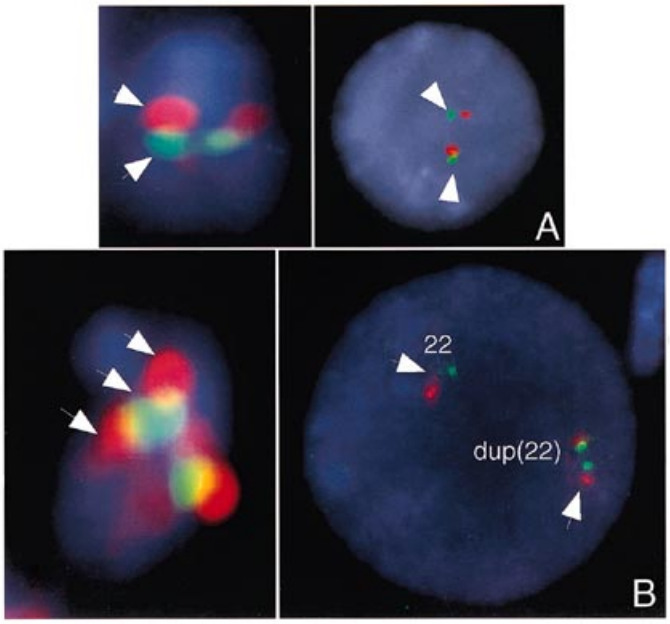

Figure 4. FISH mapping studies. Metaphase (left) and interphase (right) chromosomes were hybridized with probes derived from cosmids 70A2 (rhodamine, red) and 48C12 (fluorescein, green) that map to 22q11 (Fig. 1) in normal cells (**A**) and BM495 (**B**). Arrows in the left panels demarcate cosmid signals; arrows in the right panels demarcate the two copies of chromosome 22.

DISCUSSION

Mechanism of 22q11 rearrangements in congenital anomaly disorders

We propose the following models shown in Figure 6 to explain how the LCR22s could mediate duplications and deletions of the 22q11 region by homologous recombination mechanisms. Both inter- and intra-chromosomal homologous recombination events mediate the 3 Mb deletion in association with VCFS/DGS (25,28). We hypothesize that meiotic homologous recombination events between LCR22s mediate these chromosomal rearrangements (Fig. 6A and B). The proximal and distal 3 Mb LCR22s are 250 kb in size and share a 200 kb direct repeat that is highly homologous (25). The 200 kb interval consists of a region containing five genes or pseudogenes that are flanked by inverted sub-repeats composed of anonymous genomic sequences (Fig. 1). It is possible that the chromosomes misalign at the LCR22s because they contain large regions of sequence homology. The 200 kb interval provides the means to generate both a duplication and a deletion by an inter-chromosomal recombination mechanism (Fig. 6A and D) or a deletion by an intra-chromosomal mechanism (Fig. 6B). In addition to the 3 Mb deletion, similar mechanisms between the LCR22s at the 1.5 Mb breakpoint intervals may mediate the rearrangements leading to the 1.5 Mb deletion as well. It is of interest that a patient with a few, but not all, of the clinical findings of VCFS/DGS has been described recently to have a distal deletion flanked by *D22S1709* and *D22S308* proximally, and *D22S257* and *D22S301*, distally (Fig. 5) (42). The proximal breakpoint of this patient occurs in the same interval as the common 3 Mb distal deletion breakpoint in VCFS/DGS

Downloaded from https://academic.oup.com/hmg/article/8/7/1157/609436 by guest on 21 August 2022

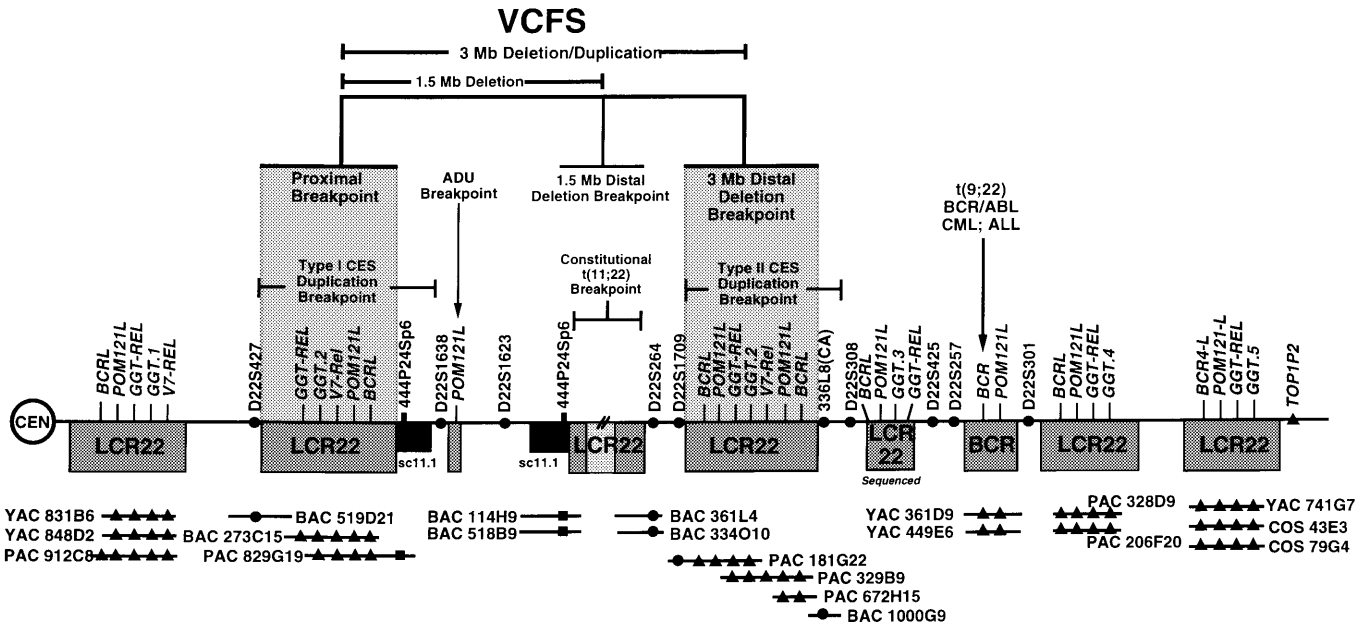

Figure 5. LCR22s and rearrangements associated with human disorders on 22q11. The eight LCR22 loci, indicated as gray boxes, are ordered from the centromere to telomere, left to right, on the line representing chromosome 22q11. Each of the human disorders resulting from 22q11 rearrangements is shown. The clones that are positive by PCR for selected gene-based (triangles), monomorphic (squares) and polymorphic (circles) markers are indicated below the line. The different GGT family members (40) that comprise each LCR22 are shown. The two *sc11.1* loci are shown as black boxes. The order of the genes on the two 3 Mb LCR22-*GGT.*2s (Fig. 1) as well as the LCR22-*GGT.3* are indicated. The order of the genes within the other LCR22s has not been established.

patients, and the distal breakpoint in this patient occurs in the same interval that harbors the LCR22 containing the *BCR* gene disrupted in tumor cells that carry the balanced t(9;22) translocation (1,43–46). Therefore, it is possible that the novel deletion that occurred in this patient is mediated by homologous recombination between the two LCR22s.

To generate the CES bisatellited chromosome, we propose that a meiotic inter-chromosomal recombination event may occur between inverted regions within the LCR22s, such as the inverted sub-repeats (Figs 1 and 6C). To generate the 3 Mb interstitial duplication present in BM498, BM496 and BM498, we propose a two-step mechanism. The first step is a meiotic inter-chromosomal recombination event between the proximal and distal LCR22s which generates the duplication of the 3 Mb region, an event supported by haplotype results (Fig. 4). A second intra-chromosomal event between inverted regions of the proximal and distal LCR22 of the duplicated segment would then result in an inversion of the region (Fig. 6D). Although the likelihood that two independent events occurred in a single meiosis is low, it is possible that the first event predisposed the chromosome to a second rearrangement so that the two events occurred in succession.

The molecular basis of the clinical phenotype of patient BM495 is intriguing. As mentioned above, the patient is affected with moderate developmental delay but does not have VCFS/DGS. The mother and grandmother, BM496 and BM498, respectively, have the same chromosome rearrangement, but are apparently normal. It is possible that a duplication of the 3 Mb region is associated with a milder phenotype that would result in under-diagnosis of this rearrangement in the general population. An alternative possibility is that the phenotype of BM495 results from other genetic or non-genetic causes and is unrelated to the rearrangement on 22q11. It will be necessary to identify additional individuals with this duplication in order to distinguish between these two possibilities.

Somatic rearrangements of 22q11 and malignant disease

Somatic rearrangements of 22q11 are associated with malignant disorders. The balanced t(9;22) breakpoint translocation results in the generation of an abnormal BCR–ABL fusion protein and results in ALL and CML (43–45). The t(9;22) breakpoint junctions from several tumors have been cloned and sequenced (46). It is possible that the chromosome rearrangements are mediated by specific elements within the *BCR* and/or *ABL* gene that could confer susceptibility to rearrangements. There is no evidence of specific sequence elements that could mediate such an event; however, the t(9;22) breakpoints appear to be associated with highly repetitive sequences that include Alu family members (47).

A balanced t(17;22) translocation in a family with neurofibromatosis type 1 has recently been identified (48). The breakpoint junction was cloned and sequenced to determine the mechanism of the rearrangement. We examined the sequences that are disrupted on chromosome 22q11 and found that they correspond to the marker 599O20SP6 that maps to the inverted sub-repeats within the proximal and distal LCR22s (25). This finding directly implicates the LCR22 sequences in rearrangements associated with malignancy. Additionally, loss of heterozygosity studies of an ependymoma-associated constitutional t(1;22)(p22;q11.2) translocation showed that the breakpoint on 22q11 is between *ARVCF* and *D22S264* (49), the region containing the 1.5 Mb distal LCR22 (Figs 1 and 5). Finally, there has been one report demonstrating a correlation between carriers of the constitutional t(11;22) translocation and breast cancer (50). These results suggest that the LCR22s may confer susceptibility

Downloaded from https://academic.oup.com/hmg/article/8/7/1157/609436 by guest on 21 August 2022

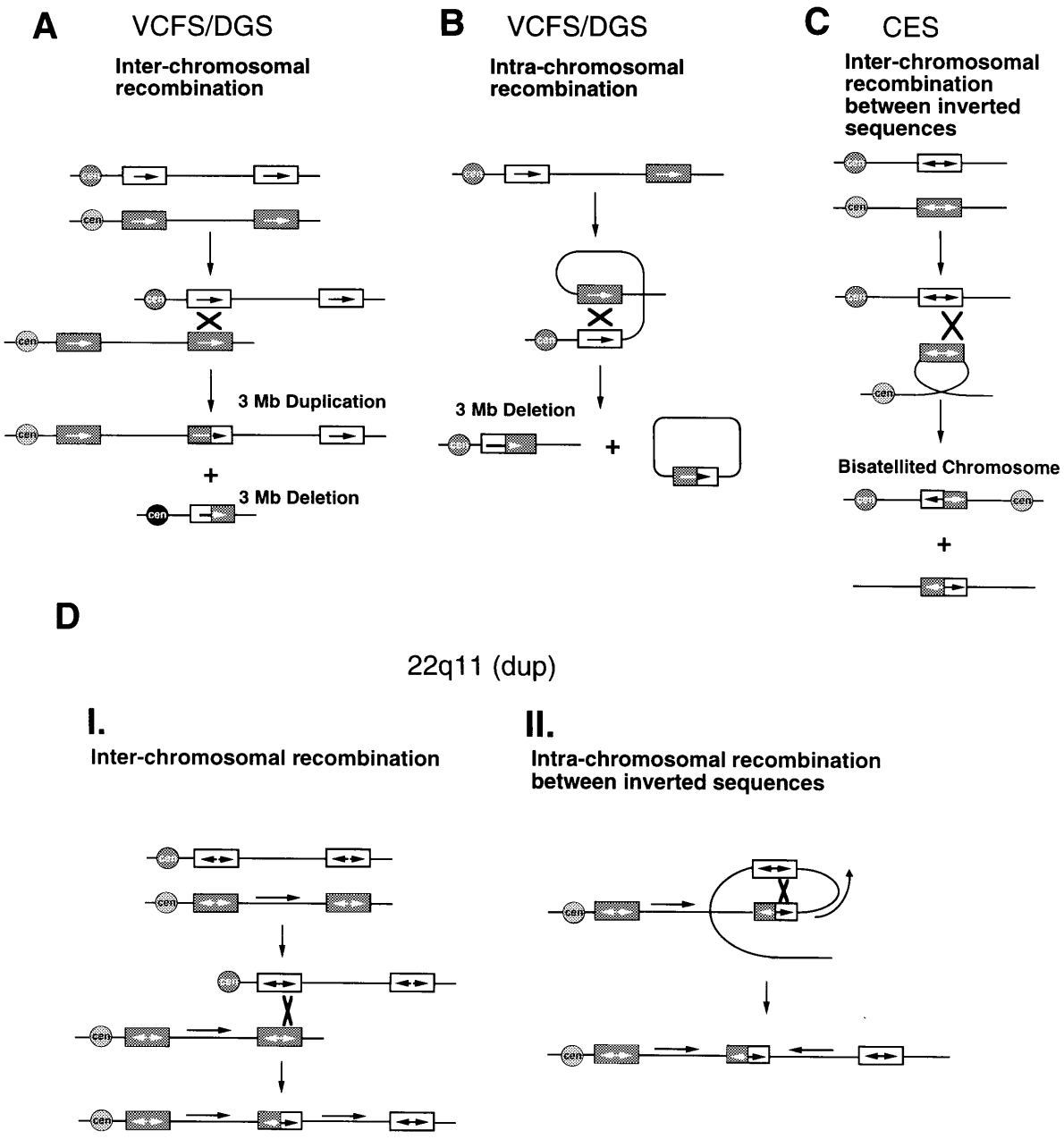

Figure 6. Models for homologous recombination events involving the LCR22s. (**A**) Inter-chromosomal homologous recombination between the 3 Mb LCR22s of two homologous chromosomes 22 could generate both the 3 Mb deletion of VCFS patients and the duplication as in BM495. (**B**) Intra-chromosomal recombination between the 3 Mb LCR22s can generate a VCFS/DGS deletion by excision of an inter-chromatidal loop. The other product of this recombination event is a hypothetical circular chromosome lacking a centromere. (**C**) Homologous recombination between the inverted sequences of the proximal and/or distal LCR22 could generate the bisatellited chromosome 22 present in CES patients. The other product of the recombination would not contain a centromere. (**D**) I: inter-chromosomal homologous recombination between directly repeated sequences in the proximal and distal 3 Mb LCR22s is followed by, II, intra-chromosomal recombination between inverted sequences generating an inversion. This type of event could explain the results obtained by analysis of BM495.

to rearrangements associated with malignant disease as well as somatic rearrangements.

The basis of other chromosome rearrangement disorders

Low copy repeats present on other chromosomes have been implicated in rearrangements leading to different chromosomal disorders (reviewed in ref. 51). The two most well characterized chromosomal disorders are Charcot–Marie–Tooth disease type 1A/hereditary neuropathy with liability to pressure palsies (CMT1A/HNPP), two peripheral neuropathies associated with a duplication/deletion, respectively, of the same region on chromosome 17p11.2–12 (52,53). Adjacent to the CMT1A/HNPP interval, but unrelated in sequence, is the Smith–Magenis syndrome (SMS) region (54,55). Three copies of a 200 kb repeat map to the interval (56). The two outer repeats are implicated in mediating the 5 Mb common deletion

Downloaded from https://academic.oup.com/hmg/article/8/7/1157/609436 by guest on 21 August 2022

associated with the disorder (56). Low copy repeats have also been localized to the breakpoint regions of other rearrangement disorders, including Williams–Beuren syndrome on 7q11.23 (57,58), as well as Prader–Willi and Angelman syndromes on 15q11–13 (59,60).

A 24 kb duplication of sequences flanking a 1.5 Mb region has been implicated in mediating the rearrangements associated with CMT1A/HNPP on chromosome 17 (61). A recombination hotspot has been identified as a 557 bp region of sequence identity between the two repeats (62). Interestingly, a *mariner* transposon-like element (63) was found near the hotspot, suggesting that strand exchange could be mediated by a transposase (62,64). It would be of interest to determine whether a similar recombination hotspot is present in the LCR22s or whether the breakpoints occur randomly throughout the repeat, at stretches of sequence identity (65,66). To determine if a recombination hotspot exists and why the 3 Mb LCR22s are particularly prone to rearrangements, it will be necessary to sequence the repeats and clone the site of chromosome breakage and strand exchange in individual patients. Furthermore, understanding the organization of each LCR22 may provide clues as to why certain types of rearrangements are more prevalent in the population. It will also be valuable to ascertain whether certain individuals are more prone to chromosome rearrangements based on sequence polymorphisms or chromosomal haplotypes.

MATERIALS AND METHODS

Somatic cell hybrid cell lines

The method used to generate hamster–human somatic hybrid cell lines from patients BM41, BM293 and BM308 has been described previously (18). Hamster–human somatic cell hybrids were generated from the patients ADU and BM15 as described (18). Briefly, polyethylene glycol 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 and 22. The selection for chromosome X is necessary since there is no efficient positive selection system available for retention of chromosome 22. The positive clones containing chromosome 22 were expanded and genotyped with genetic markers spanning the chromosome 22q11 region to confirm the integrity the clones. For the balanced t(2;22) translocation patient ADU (19), three different cell lines were generated that contained chromosome 22 sequences, for the der(2), der(22) and normal copy of chromosome 22. Clones containing chromosome 22 were expanded further and DNA was purified (Puregene kit; Gentra, Minneapolis, MN). The somatic hybrid cell line carrying the deleted copy of BM15 was verified by genotype analysis and expanded as for patient ADU. Once the integrity of the clones was verified, PCR was performed on 50 ng of template DNA as described previously (29).

Preparation of DNA from the BM495 family and haplotype analysis

Genomic DNA was prepared from 5 ml of peripheral blood obtained from BM495 and family members with their informed consent (Internal Review Board approved; Human Genetics Program at Albert Einstein College of Medicine), using the Puregene protocol (Gentra) as described by Morrow *et al*. (29). A maximum of 15 highly polymorphic genetic markers from *D22S420* to *D22S257* were used for genotyping each individual as described (29).

Fluorescence *in situ* hybridization (FISH)

The cosmid clones 70A2 and 48C12 were isolated from the gridded LL22NC03 cosmid library (P. De Jong, Roswell Park Cancer Institute, NY) and were used for FISH mapping studies. FISH was performed as described by Rao *et al*. (67). Briefly, the cosmids were differentially labeled with biotin-14-dATP (Gibco BRL, Grand Island, NY) and digoxigenin-11-dUTP (Boehringer Mannheim, Indianapolis, IN), respectively, by the nick translation method. The probes (200 ng) were dissolved in 10 µl of hybridization mixture (50% formamide, 10% dextran sulfate and 2× SSC) in the presence of 5 µg of unlabeled human Cot-1 DNA (Gibco BRL) and hybridized overnight at 37ºC. Post-hybridization washes were performed in 2× SSC at 45°C for 10 min. The digoxigenin- and biotin-labeled probes were detected using a mixture of rhodamine-labeled anti-digoxigenin and fluorescein-labeled avidin (Ventana Medical Systems, Tucson, AZ). To identify the chromosomes, they were counterstained with 4',6-diaminido-2-phenylindole dihydrochloride (DAPI). Fluorescent hybridization signals and DAPI staining patterns were captured with an IMAC-CCD-S36 camera attached to a Nikon Optiphot-2 microscope and processed using ISIS (Insitu Imaging System) image processing software (Metasystems).

ACKNOWLEDGEMENTS

We thank Drs Raju Kucherlapati, Arthur Skoultchi, Anne Puech, Bruno Saint-Jore and Steven Somlo for their helpful suggestions. We thank the families for their participation in the study. B.E.M. is supported by NIH grant PO-1 HD34980-02, American Heart Association Grant-in-Aid, and Investigatorship and March of Dimes Grant (FY98-0414). L.E. is supported by NIH grant T32 CA09060. B.F. is supported by a grant from Deutsche Forschungsgemeinschaft. R.J.S. is supported by NIH grant PO-1 HD34980-02.

REFERENCES

1. Nowell, P.C. and Hungerford, D.A. (1960) A minute chromosome in human chronic granulocytic leukemia. *Science*, **132**, 1497–1499.
2. Emanuel, B.S., Selden, J.R., Wang, E., Nowell, P.C. and Croce, C.M. (1984) *In situ* hybridization and translocation breakpoint mapping. I. Non-identical 22q11 breakpoints for the t(9;22) of CML and the t(8;22) of Burkitt lymphoma. *Cytogenet. Cell Genet.*, **38**, 127–131.
3. Davis, M., Malcolm, S. and Rabbitts, T.H. (1984) Chromosome translocation can occur on either side of the c-*myc* oncogene in Burkitt lymphoma cells. *Nature*, **308**, 286–288.
4. Schinzel, A., Schmid, W., Fraccaro, M., Tiepolo, L., Zuffardi, O., Opitz, J.M., Lindsten, J., Zetterqvist, P., Enell, H., Baccichetti, C., Tenconi, R. and Pagon, R.A. (1981) The 'cat eye syndrome': dicentric small marker chromosome probably derived from a no. 22 (tetrasomy 22pter>q11) associated with a characteristic phenotype. *Hum. Genet.*, **57**, 148–158.
5. Zackai, E.H. and Emanuel, B.S. (1980) Site-specific reciprocal translocation t(11;22)(q23;q11), in several unrelated families with 3:1 meiotic disjunction. *Am. J. Med. Genet.*, **7**, 507–521.
6. Shprintzen, R.J., Goldberg, R.B., Lewin, M.L., Sidoti, E.J., Berkman, M.D., Argamaso, R.V. and Young, D. (1978) A new syndrome involving

Downloaded from https://academic.oup.com/hmg/article/8/7/1157/609436 by guest on 21 August 2022

cleft palate, cardiac anomalies, typical facies, and learning disabilities: velo-cardio-facial syndrome. *Cleft Palate J.*, **15**, 56–62.
7. DiGeorge, A. (1965) A new concept of the cellular basis of immunity. *J. Pediatr.*, **67**, 907.
8. Emanuel, B.S., Nowell, P.C., McKeon, C., Croce, C.M. and Israel, M.A. (1986) Translocation breakpoint mapping: molecular and cytogenetic studies of chromosome 22. *Cancer Genet. Cytogenet.*, **19**, 81–92.
9. Budarf, M.L., McDermid, H.E., Sellinger, B. and Emanuel, B.S. (1991) Isolation and regional localization of 35 unique anonymous DNA markers for human chromosome 22. *Genomics*, **10**, 996–1002.
10. Budarf, M.L., Eckman, B., Michaud, D., McDonald, T., Gavigan, S., Buetow, K.H., Tatsumura, Y., Liu, Z., Hilliard, C., Driscoll, D., Goldmuntz, E., Meese, E., Zwarthoff, E.C., Williams, S., McDermid, H., Dumanski, J.P., Biegel, J., Bell, C.J. and Emanuel, B.S. (1996) Regional localization of over 300 loci on human chromosome 22 using a somatic cell hybrid mapping panel. *Genomics*, **15**, 275–288.
11. Mears, A.J., Duncan, A.M.V., Budarf, M.L., Emanuel, B.S., Sellinger, B., Siegel-Bartelt, J., Greenberg, C.R. and McDermid, H.E. (1994) Molecular characterization of the marker chromosome associated with cat eye syndrome. *Am. J. Hum. Genet.*, **55**, 134–142.
12. Mears, A.J., el-Shanti, H., Murray, J.C., McDermid, H.E. and Patil, S.R. (1995) Minute supernumerary ring chromosome 22 associated with cat eye syndrome: further delineation of the critical region. *Am. J. Hum. Genet.*, **57**, 667–673.
13. McTaggart, K.E., Budarf, M.L., Driscoll, D.A., Emanuel, B.S., Ferreira, P. and McDermid H.E. (1998) Cat eye syndrome chromosome breakpoint clustering: identification of two intervals also associated with 22q11 deletion syndrome breakpoints. *Cytogenet. Cell Genet.*, **81**, 222–228.
14. Fraccaro, M., Lindsten, J., Ford, C.E. and Iselius, L. (1980) The 11q;22q translocation: a European collaborative analysis of 43 cases. *Hum. Genet.*, **56**, 21–51.
15. Funke, B., Edelmann, L., McCain, N., Pandita, R., Ferreira, J., Merscher, S., Zohouri, M., Cannizzaro, L., Shanske, A. and Morrow B.E. (1999) Der(22) syndrome and velo-cardio-facial syndrome/DiGeorge syndrome share a 1.5 Mb region of overlap on chromosome 22q11. *Am. J. Hum Genet.*, **64**, 747–758.
16. Burn, J. and Goodship, J. (1996) Congenital heart disease. In Rimoin, D.L., Connor, J.M. and Pyeritz, R.E. (eds), *Emery and Rimoin's Principles and Practice of Medical Genetics.* 3rd edn. Vol. 1, pp. 767–828.
17. Papolos, D.F., Faedda, G.L., Veit, S., Goldberg, R., Morrow, B., Kucherlapati, R. and Shprintzen, R.J. (1996) Bipolar spectrum disorders in patients diagnosed with velo-cardio-facial syndrome: does a hemizygous deletion of chromosome 22q11 result in bipolar affective disorder? *Am. J. Psychiatry*, **153**, 1541–1547.
18. Carlson, C., Sirotkin, H., Pandita, R., Goldberg, R., McKie, J., Wadey, R., Patanjali, S.R., Weissman, S.M., Anyane-Yeboa, K., Warburton, D., Scambler, P., Shprintzen, R., Kucherlapati, R. and Morrow, B.E. (1997) Molecular definition of 22q11 deletions in 151 velo-cardio-facial syndrome patients. *Am. J. Hum Genet.,* **61**, 620–629.
19. Augusseau, S., Jouk, S., Jalbert, P. and Prieur, M. (1986) DiGeorge syndrome and 22q11 rearrangements. *Hum. Genet.*, **74**, 206.
20. Lindsay, E.A., Goldberg, R., Jurecic, V., Morrow, B., Carlson, C., Kucherlapati, R.S., Shprintzen, R.J. and Baldini, A. (1995) Velo-cardio-facial syndrome: frequency and extent of 22q11 deletions. *Am. J. Med. Genet.*, **57**, 514–522.
21. Kurahashi, H., Nakayama, T., Osugi, Y., Tsuda, E., Masuno, M., Imaizumi, K., Kamiya, T., Sano, T., Okada, S. and Nishisho, I. (1996) Deletion mapping of 22q11 in CATCH22 syndrome: identification of a second critical region. *Am. J. Hum. Genet.*, **58**, 1377–1381.
22. Levy, A., Demczuk, S., Aurias, A., Depetris, D., Mattei, M. and Philip, N. (1995) Interstitial 22q11 microdeletion excluding the ADU breakpoint in a patient with DiGeorge syndrome. *Hum. Mol. Genet.*, **4**, 2417–2419.
23. O'Donnell, H., McKeown, C., Gould, C., Morrow, B. and Scambler, P. (1997) Detection of an atypical 22q11 deletion that has no overlap with the DiGeorge syndrome critical region. *Am. J. Hum. Genet.*, **60**, 1544–1548.
24. Holmes, S.E., Riazi, M.A., Gong, W., McDermid, H.E., Sellinger, B.T., Hua, A., Chen, F., Wang, Z., Zhang, G., Roe, B., Gonzalez, I., McDonald-McGinn, D.M., Zackai, E., Emanuel, B.S. and Budarf, M.L. (1997) Disruption of the clathrin heavy chain-like gene (*CLTCL*) associated with features of DGS/VCFS: a balanced (21;22)(p12;q11) translocation. *Hum. Mol. Genet.*, **6**, 357–367.
25. Edelmann, L., Pandita, R.K. and Morrow, B.E. (1999) Low copy repeats mediate the common 3 Mb deletion in velo-cardio-facial syndrome patients on 22q11. *Am. J. Hum. Genet.*, **64**, 1076–1086.
26. Halford, S., Lindsay, E., Nayudu, M., Carey, A.H., Baldini, A. and Scambler, P.J. (1993) Low-copy-number repeat sequences flank the DiGeorge/velo-cardio-facial syndrome loci at 22q11. *Hum. Mol. Genet.*, **2**, 191–196.
27. Lindsay, E.A., Halford, S., Wadey, R., Scambler, P.J. and Baldini, A. (1993) Molecular cytogenetic characterization of the DiGeorge syndrome region using fluorescence *in situ* hybridization. *Genomics*, **17**, 403–407.
28. Baumer, A., Dutly, F., Balmer, D., Riegel, M., Tukel, T., Krajewska-Walasek, M. and Schinzel, A.A. (1998) High level of unequal meiotic crossovers at the origin of the 22q11.2 and 7q11.23 deletions. *Hum. Mol. Genet.*, **7**, 887–894.
29. Morrow, B., Goldberg, R., Carlson, C., Das Gupta, R., Sirotkin, H., Collins, J., Dunham, I., O'Donnell, H., Scambler, P., Shprintzen, R. and Kucherlapati, R. (1995) Molecular definition of the 22q11 deletions in velo-cardio-facial syndrome. *Am. J. Hum. Genet.*, **56**, 1391–1403.
30. Osoegawa, K., Woon, P.Y., Zhao, B., Frengen, E., Tateno, M., Catanese, J.J. and de Jong, P.J. (1998) An improved approach for construction of bacterial artificial chromosome libraries. *Genomics*, **52**, 1–8.
31. Budarf, M.L., Collins, J., Gong, W., Roe, B., Wang, Z., Bailey, L.C., Sellinger, B., Michaud, D., Driscoll, D. and Emanuel, B.S. (1995) Cloning a balanced translocation associated with DiGeorge syndrome and identification of a disrupted candidate gene. *Nature Genet.*, **10**, 269–278.
32. Demczuk, S., Aledo, R., Zucman, J., Delattre, O., Desmaze, C., Dauphinot, L., Jalbert, P., Rouleau, G.A., Thomas, G. and Aurias, A. (1995) Cloning of a balanced translocation breakpoint in the DiGeorge syndrome critical region and isolation of a novel potential adhesion receptor gene in its vicinity. *Hum. Mol. Genet.*, **4**, 551–558.
33. Heisterkamp, N., Rajpert-De Meyts, E., Uribe, L., Forman, H.J. and Groffen, J. (1991) Identification of a human gamma-glutamyl cleaving enzyme related to, but distinct from, gamma-glutamyl transpeptidase. *Proc. Natl Acad. Sci. USA*, **88**, 6303–6307.
34. Figlewicz, D.A., Delattre, O., Guellaen, G., Krizus, A., Thomas, G., Zucman, J. and Rouleau, G.A. (1993) Mapping of human gamma-glutamyl transpeptidase genes on chromosome 22 and other human autosomes. *Genomics*, **17**, 299–305.
35. Ruegg, C.L., Rivas, A., Madani, N.D., Zeitung, J., Laus, R. and Engleman, E.G. (1995) V7, a novel leukocyte surface protein that participates in T cell activation. II. Molecular cloning and characterization of the V7 gene. *J. Immunol.*, **154**, 4434–4443.
36. Soderqvist, H., Jiang, W.Q., Ringertz, N. and Hallberg, E. (1996) Formation of nuclear bodies in cells overexpressing the nuclear pore protein POM121. *Exp. Cell Res.*, **225**, 75–84.
37. Croce, C.M., Huebner, K., Isobe, M., Fainstain, E., Lifshitz, B., Shtivelman, E. and Canaani, E. (1987) Mapping of four distinct *BCR*-related loci to chromosome region 22q11: order of *BCR* loci relative to chronic myelogenous leukemia and acute lymphoblastic leukemia breakpoints. *Proc. Natl Acad. Sci. USA*, **84**, 7174–7178.
38. Heisterkamp, N.and Groffen, J. (1988) Duplication of the *bcr* and gamma-glutamyl transpeptidase genes. *Nucleic Acids Res.*, **16**, 8045–8056.
39. Courtay, C., Heisterkamp, N., Siest, G. and Groffen, J. (1994) Expression of multiple gamma-glutamyltransferase genes in man. *Biochem. J.*, **297**, 503–508.
40. Collins, J.E., Mungall, A.J., Badcock, K.L., Fay, J.M. and Dunham, I. (1997) The organization of the gamma-glutamyl transferase genes and other low copy repeats in human chromosome 22q11. *Genet. Res.*, **7**, 522–531.
41. Kawasaki, K., Minoshima, S., Nakato, E., Shibuya, K., Shintani, A., Schmeits, J.L., Wang, J. and Shimizu, N. (1997) One-megabase sequence analysis of the human immunoglobulin lambda gene locus. *Genome Res.*, **7**, 250–261.
42. Rauch, A., Pfeiffer, R.A., Leipold, G., Singer, H., Tigges, M. and Hofbeck, M. (1999) A novel 22q11.2 microdeletion in DiGeorge syndrome. *Am. J. Hum. Genet.*, **64**, 658–666.
43. de Klein, A., van Kessel, A.G., Grosveld, G., Bartram, C.R., Hagemeijer, A., Bootsma, D., Spurr, N.K., Heisterkamp, N., Groffen, J. and Stephenson, J.R. (1982) A cellular oncogene is translocated to the Philadelphia chromosome in chronic myelocytic leukaemia. *Nature*, **300**, 765–767.
44. Shtivelman, E., Lifshitz, B., Gale, R.P. and Canaani, E. (1985) Fused transcript of *abl* and *bcr* genes in chronic myelogenous leukaemia. *Nature*, **315**, 550–554.

Downloaded from https://academic.oup.com/hmg/article/8/7/1157/609436 by guest on 21 August 2022

45. Hermans, A., Heisterkamp, N., von Linden, M., van Baal, S., Meijer, D., van der Plas, D., Wiedemann, L.M., Groffen, J., Bootsma, D. and Grosveld, G. (1987) Unique fusion of *bcr* and c-*abl* genes in Philadelphia chromosome positive acute lymphoblastic leukemia. *Cell*, **51**, 33–40.
46. Chissoe, S.L., Bodenteich, A., Wang, Y.F., Wang, Y.P., Burian, D., Clifton, S.W., Crabtree, J., Freeman, A., Iyer, K., Jian, L., Ma, Y., McLaury, H.-J., Pan, H.-Q., Sarhan, O.H., Toth, S., Wang, Z., Zhang, G., Heisterkamp, N., Groffen, J. and Roe, B.A. (1995) Sequence and analysis of the human *ABL* gene, the *BCR* gene, and regions involved in the Philadelphia chromosomal translocation. *Genomics*, **1**, 67–82.
47. Jeffs, A.R., Benjes, S.M., Smith, T.L., Sowerby, S.J. and Morris, C.M. (1998) The *BCR* gene recombines preferentially with Alu elements in complex *BCR–ABL* translocations of chronic myeloid leukaemia. *Hum. Mol. Genet.*, **7**, 767–776.
48. Kehrer-Sawatzki, H., Maier, C., Moschgath, E., Elgar, G. and Krone, W. (1997) The second case of a t(17;22) in a family with neurofibromatosis type 1: sequence analysis of the breakpoint regions. *Hum. Genet.*, **99**, 237–247.
49. Rhodes, C.H., Call, K.M., Budarf, M.L., Barnoski, B.L., Bell, C.J., Emanuel, B.S., Bigner, S.H., Park, J.P. and Mohandas, T.K. (1997) Molecular studies of an ependymoma-associated constitutional t(1;22) (p22;q11.2). *Cytogenet. Cell Genet.*, **78**, 247–252.
50. Lindblom, A., Sandelin, K., Iselius, L., Dumanski, J., White, I., Nordenskjold, M. and Larsson, C. (1994) Predisposition for breast cancer in carriers of constitutional translocation 11q;22q. *Am. J. Hum. Genet.*, **54**, 871–876.
51. Lupski, J.R. (1998) Genomic disorders: structural features of the genome can lead to DNA rearrangements and human disease traits. *Trends Genet.*, **14**, 417–422.
52. Lupski, J.R., de Oca-Luna, R.M., Slaugenhaupt, S., Pentao, L., Guzzetta, V., Trask, B.J., Saucedo-Cardenas, O., Barker, D.F., Killian, J.M., Garcia, C.A., Chakravarti, A. and Patel, P.I. (1991) DNA duplication associated with Charcot–Marie–Tooth disease type 1A. *Cell*, **66**, 219–232.
53. Chance, P.F., Abbas, N., Lensch, M.W., Pentao, L., Roa, B.B., Patel, P.I. and Lupski, J.R. (1994) Two autosomal dominant neuropathies result from reciprocal DNA duplication/deletion of a region on chromosome 17. *Hum. Mol. Genet.*, **3**, 223–228.
54. Smith, A.C., McGavran, L., Robinson, J., Waldstein, G., Macfarlane, J., Zonona, J., Reiss, J., Lahr, M., Allen, L. and Magenis, E. (1986) Interstitial deletion of (17)(p11.2p11.2) in nine patients. *Am. J. Med. Genet.*, **24**, 393–414.
55. Greenberg, F., Guzzetta, V., Montes de Oca-Luna, R., Magenis, R.E., Smith, A.C., Richter, S.F., Kondo, I., Dobyns, W.B., Patel, P.I. and Lupski, J.R. (1991) Molecular analysis of the Smith–Magenis syndrome: a possible contiguous-gene syndrome associated with del(17)(p11.2). *Am. J. Hum. Genet.*, **49**, 1207–1218.
56. Chen, K.S., Manian, P., Koeuth, T., Potocki, L., Zhao, Q., Chinault, A.C., Lee, C.C. and Lupski, J.R. (1997) Homologous recombination of a flanking repeat gene cluster is a mechanism for a common contiguous gene deletion syndrome. *Nature Genet.*, **17**, 154–163.
57. Osborne, L.R., Herbrick, J.A., Greavette, T., Heng, H.H., Tsui, L.C. and Scherer, S.W. (1997) PMS2-related genes flank the rearrangement breakpoints associated with Williams syndrome and other diseases on human chromosome 7. *Genomics*, **45**, 402–406.
58. Perez Jurado, L.A., Wang, Y.K., Peoples, R., Coloma, A., Cruces, J. and Francke, U. (1998) A duplicated gene in the breakpoint regions of the 7q11.23 Williams–Beuren syndrome deletion encodes the initiator binding protein TFII-I and BAP-135, a phosphorylation target of BTK. *Hum. Mol. Genet.*, **7**, 325–334.
59. Carrozzo, R., Rossi, E., Christian, S.L., Kittikamron, K., Livieri, C., Corrias, A., Pucci, L., Fois, A., Simi, P., Bosio, L., Beccaria, L., Zuffardi, O. and Ledbetter, D.H. (1997) Inter- and intrachromosomal rearrangements are both involved in the origin of 15q11–q13 deletions in Prader–Willi syndrome. *Am. J. Hum. Genet.*, **61**, 228–231.
60. Wandstrat, A.E., Leana-Cox, J., Jenkins, L. and Schwartz, S. (1998) Molecular cytogenetic evidence for a common breakpoint in the largest inverted duplications of chromosome 15. *Am. J. Hum. Genet.*, **62**, 925–936.
61. Pentao, L., Wise, C.A., Chinault, A.C., Patel, P.I. and Lupski, J.R. (1992) Charcot–Marie–Tooth type 1A duplication appears to arise from recombination at repeat sequences flanking the 1.5 Mb monomer unit. *Nature Genet.*, **2**, 292–300.
62. Reiter, L.T., Murakami, T., Koeuth, T., Pentao, L., Muzny, D.M., Gibbs, R.A. and Lupski, J.R. (1996) A recombination hotspot responsible for two inherited peripheral neuropathies is located near a *mariner* transposon-like element. *Nature Genet.*, **12**, 288–297.
63. Jacobson, J.W., Medhora, M.M. and Hartl, D.L. (1986) Molecular structure of a somatically unstable transposable element in *Drosophila*. *Proc. Natl Acad. Sci. USA*, **83**, 8684–8688.
64. Reiter, L.T., Hastings, P.J., Nelis, E., De Jonghe, P., Van Broeckhoven, C. and Lupski, J.R. (1998) Human meiotic recombination products revealed by sequencing a hotspot for homologous strand exchange in multiple HNPP deletion patients. *Am. J. Hum. Genet.*, **62**, 1023–1033.
65. Liskay, R.M. and Stachelek, J.L. (1986) Information transfer between duplicated chromosomal sequences in mammalian cells involves contiguous regions of DNA. *Proc. Natl Acad. Sci. USA*, **83**, 1802–1806.
66. Waldman, A.S. and Liskay, R.M. (1988) Dependence of intrachromosomal recombination in mammalian cells on uninterrupted homology. *Mol. Cell. Biol.*, **8**, 5350–5357.
67. Rao, P.H., Murty, V.V.V.S., Gaidano, G., Hauptschein, R., Dalla-Favera, R. and Chaganti, R.S.K. (1993) Subregional localization of 20 single-copy loci to chromosome 6 by fluorescence *in situ* hybridization. *Genomics*, **16**, 426–430.

Downloaded from https://academic.oup.com/hmg/article/8/7/1157/609436 by guest on 21 August 2022