Human Molecular Genetics, 2003, Vol. 12, No. 15 **1823–1837**
DOI: 10.1093/hmg/ddg203

Frequent translocations occur between low copy repeats on chromosome 22q11.2 (LCR22s) and telomeric bands of partner chromosomes

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Received April 8, 2003; Revised and Accepted May 21, 2003

The chromosome 22q11.2 region is susceptible to rearrangements, mediated by low copy repeats (LCR22s). Deletions and duplications are mediated by homologous recombination events between LCR22s. The recurrent balanced constitutional translocation t(11;22)(q23;q11) breakpoint occurs in an LCR22 and is mediated by double strand breaks in AT-rich palindromes on both chromosomes 11 and 22. Recently, two cases of a t(17;22)(q11;q11) were reported, mediated by a similar mechanism (21). Except for these constitutional translocations, the molecular basis for non-recurrent, reciprocal 22q11.2 translocations is not known. To determine whether there are specific mechanisms that could mediate translocations, we analyzed cell lines derived from 14 different individuals by genotyping and FISH mapping. Somatic cell hybrid analysis was carried out for four cell lines. In five cell lines, the translocation breakpoints occurred in the same LCR22 as for the t(11;22) translocation, suggesting that similar molecular mechanisms are responsible. An additional three occurred in other LCR22s, and six were in non-LCR22 regions, mostly in the proximal half of the 22q11.2 region. The translocation breakpoints on the partner chromosomes were all located in the telomeric bands, proximal to the most telomeric unique sequence probe, in eight cell lines and distal to those loci in six. Therefore, several of the breakpoints were found to occur in the vicinity of highly dynamic regions of the genome, 22q11.2 and telomeric bands. We hypothesize that these regions are more susceptible to breakage and repair, resulting in translocations.

INTRODUCTION

The chromosome 22q11.2 region is susceptible to constitutional rearrangements associated with three different congenital anomaly disorders, velo-cardio-facial syndrome/DiGeorge syndrome (VCFS/DGS; MIM192430/MIM188400), cat-eye syndrome (CES; MIM 115470) and der(22) syndrome. VCFS/DGS, the most common of the three, has an estimated frequency of one in 4000–5600 live births (1,2). Most patients with this syndrome have a hemizygous 3 Mb deletion (3), some possess a 1.5 Mb deletion due to a nested distal deletion breakpoint, and a few rare patients have unique deletion breakpoints (3–11). CES patients carry a supernumerary bisatellited chromosome 22 leading to a trisomy or tetrasomy of the 22pter-q11 region (12,13). Der(22) syndrome patients have a partial trisomy for the 11q23-qter and 22pter-q11

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Table 1. Cell lines with 22q11.2 translocations

Cell line	Translocation	Diagnosis
Balanced		
GM05878[a]	46, XY, t(10;22)(10pter>10q26::2q11.2>22qter)	Normal[d]
GM07511	46, XY/46, XY, t(14;22)(14pter>14q32.3::22q11.2>22qter)	Aden. Polyposis of colon
BM798	46, XX, t(15;22)(15pter>15q26.1::22q11>22qter)	Malrotation of duodenum, absent left kidney
BM819	46, XX, t(16;22)(16pter>16q24.2::22q11.2>22qter)	VCFS/DGS related, velopharyngeal incompetence, speech and language delay
GM02324	46, XX, t(16;22)(16qter>16p13.11::22q11.2>22qter)	Normal[e]
GM00119	46, XX, t(17;22)(17qter>17p13::22q11>22qter)	Normal
GM03197	46, XX, inv(5)(pter>p13::q13>p13::q13>qter) t(17;22)(17qter>17p13::22q11>22qter)	Normal
Unbalanced		
BM510	45, XY,-1,-22,+t(1;22)(1qter>1p36::22q11>22qter)	VCFS/DGS
GM03577	45, XY,-3,-22,+t(3;22)(3pter>3q29::22q11>22qter)	Absent thymus and parathyroid glands, cleft lip and palate, congenital heart disease
GM05401[a]	45, XY,-4,-22,+t(4;22)(4pter>4q35::22q11>22qter)	Partial DiGeorge
GM00980[b]	45, XX,-11,-22,+t(11;22)(11pter>11q25::22q11>22qter)	VCFS/DGS related, developmental delay, persistent anemia, small stature, microcephaly, low set ears, flattened nose, pulmonic stenosis, double aortic arch, possible atrial septal defect and mental retardation
GM06526	45, XY,-13,-22,+t(13;22)(13pter>13q34::22q11.2>22qter)	N/A
BM58[c]	45, XY,-18,-22,+t(18;22)(18qter>18p11.31::22q11>22qter)	VCFS/DGS
GM11327	45, XX,-21,-22,+t(21;22)(21pter>21q22::22q11>22qter)	Small for gestational age, hypoplastic lungs, hydrocephalus, congenital heart disease

[a]Cannizzaro *et al.* (65); Desmaze *et al.* (22); Lindsay *et al.* (76).
[b]Kurahashi *et al.* (74); Fu *et al.* (75); Lindsay *et al.* (76).
[c]Funke *et al.* (16).
[d]Unbalanced child diagnosed with DGS.
[e]Unbalanced daughter diagnosed with anophthalmia, congenital heart defect, increased simian crease, asymmetrical ear position.

regions, due to 3 : 1 non-disjunction of a parental t(11;22)(q23;q11).

The regions of chromosome breakage for all three disorders occur in blocks of low copy repeats (LCR22s) (6,14,15). VCFS/DGS and CES share a common proximal rearrangement breakpoint in the most centromeric LCR22, LCR22-2. The VCFS/DGS 1.5 Mb distal deletion breakpoint and the constitutional t(11;22) translocation breakpoint are localized to the next LCR22, LCR22-3a (16). The t(11;22) recurrent translocation is mediated by non-homologous recombination events between AT-rich palindromes on 11q23 and 22q11.2 (17–19). Recently a constitutional t(17;22) translocation has been identified in two unrelated individuals and the mechanism is similar to that of the t(11;22) translocation (17,20,21). The VCFS/DGS 3 Mb distal deletion breakpoint, occurring in 90% of deleted patients, as well as a second CES duplication breakpoint, occur in the more telomeric LCR22, LCR22-4 (15). VCFS/DGS and CES rearrangements probably result from homologous recombination events between LCR22s, while the t(11;22) and t(17;22) are mediated by non-homologous events (13,15,16).

The 22q11.2 region is also susceptible to many non-recurrent rearrangements. Many individuals have been ascertained with both balanced and unbalanced translocations that disrupt the 22q11.2 region. Except for the recurrent constitutional t(11;22) and t(17;22) translocations (17–21), the molecular basis of sporadic 22q11.2 translocations is not known. The 22q11.2 region serves as a starting point to determine the mechanisms that result in translocations. In this report, we examine the derivative chromosomes from 14 unrelated subjects harboring translocations with breakpoints in 22q11.2. We define the breakpoint regions on 22q11.2 and the breakpoint intervals on the partner chromosomes to determine whether there are similar genomic structural features and potential common mechanisms resulting in these rearrangements.

RESULTS

Characterization of chromosome 22 breakpoints by FISH mapping

We performed mapping studies on cell lines from cell lines with 22q11.2 rearrangements (Table 1) to determine the location of the chromosome breakpoints. Seven cell lines carried balanced translocations (BM798, BM819, GM05878, GM03197, GM02324, GM07511 and GM00119) and seven cell lines carried unbalanced translocations (BM510, GM00980, GM05401, BM58, GM03577, GM06526 and GM11327). Fluorescence *in situ* hybridization (FISH) mapping, using a commercial probe, was performed on each cell line (N25, Oncor; data not shown). The chromosome 22q11.2 region spans a 7.48 Mb interval (from AC005300 to AP000359; http://genome.ucsc.edu) and blocks of LCR22s comprise ~700 kb or roughly 10% of the interval. To determine whether the breakpoints occurred in the vicinity of LCR22s or in other sequences, we genotyped cell lines with unbalanced translocations with single nucleotide and dinucleotide polymorphic markers (Fig. 1). We found that the position of the breakpoints varied, but three were in the vicinity of

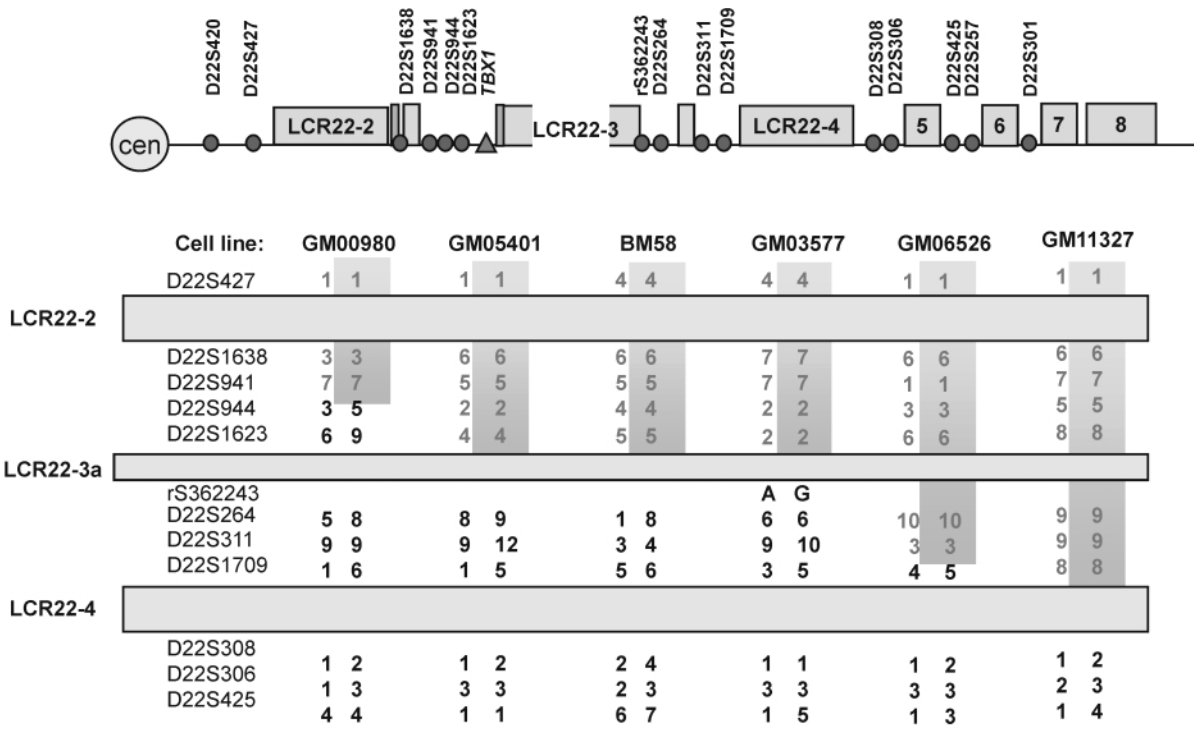


Figure 1. Genotype analysis of cell lines with unbalanced 22q11.2 translocations. The position of genetic markers (circles) and the gene, *TBX1* are indicated on the map of the LCR22s (not shown to scale) on chromosome 22q11.2. LCR22-3a contains an uncloned region, indicated by an open box. The genetic markers shown above the line representing chromosome 22q11.2 were used to perform genotype analysis on DNA isolated from the cell lines listed. The map position of the three LCR22s, LCR22-2, LCR22-3a and LCR22-4 are indicated. The boxes surrounding the genotypes in the interval that has a putative hemizygous deletion. One single nucleotide polymorphic genetic marker, rS362243 was used to narrow the breakpoint in the line GM03577. GM00980 has a breakpoint between D22S941 and D22S944; GM05401 has a breakpoint between D22S1623 and D22S264; BM58 has a breakpoint between D22S1623 and D22S264; GM03577 has a breakpoint between D22S1623 and rS62243; GM06526 has a breakpoint between D22S311 and D22S1709; GM11327 has a breakpoint between D22S1709 and D22S308.

LCR22-3a, the same LCR22 that mediates the t(11;22) and t(17;22) translocation (Fig. 1). FISH mapping was then performed with clones spanning the 22q11.2 region in the cell lines with balanced and unbalanced translocations (Table 2; Fig. 2A).

Balanced translocations on 22q11.2

We first examined the cell lines containing balanced 22q11.2 translocations by FISH mapping. For FISH mapping, we used probes that were located within 25 kb of each of the LCR22s (Fig. 2A). The most centromeric breakpoint occurred in a cell line carrying a balanced t(17;22) translocation, termed GM03197. The breakpoint on chromosome 22q11.2 occurred between two probes, CTD677F7 and PAC493F17 (Fig. 2B and C). The 3′ end of BAC clone CTD677F7 maps 10 kb centromeric to LCR22-2. The distal probe, 493F17, maps 4 kb distal to the LCR22-2 interval [including sc11.1 locus, a duplicated element containing DGCR6 and PRODH (14); Fig. 2B]. These results suggest that the breakpoint is in LCR22-2, the same LCR22 that mediates the common VCFS/DGS and CES duplication breakpoints. The cell line, GM05878, had a balanced t(10;22) translocation (Table 1) and its translocation breakpoint on 22q11.2 was between probes F5 and BAC361L10 (Fig. 2A), in non-LCR22 sequences (data not shown). The breakpoints for cell lines BM819 and GM00119 (Table 1) mapped distal to two overlapping PACs, PAC201M18 and PAC444P24 (GenBank accession no. AC007663) and proximal to PAC353B13. Representative FISH mapping experiments for cell lines with breakpoints in LCR22-3a are shown in Figure 3. Clone PAC201M18 maps 25 kb centromeric to LCR22-3a and PAC444P24 maps 1 kb within LCR22-3a. Both were missing in the derivative chromosomes 16 and 17, respectively (Fig. 3B). Clone PAC353B13 maps 16 kb telomeric to LCR22-3a (16) and it was present in the derivative chromosomes. This placed the breakpoints in the immediate vicinity of LCR22-3a (Fig. 3A).

The three cell lines, GM07511B, BM798 and GM02324 (Table 1), had translocations that occurred more telomeric to the common 3 Mb deletion interval (BM798, Figs 1 and 4; GM07511, Fig. 4; GM02324, data not shown). The mosaic cell line, GM07511B, had a balanced t(14;22) translocation in the vicinity of LCR22-5, between two overlapping clones, cosmid 61E11 (76; Fig. 4B) and RP11 BAC 757F24 (3′ end of clone has 3 kb overlap with LCR22-5; data not shown) and BAC 865E9 (data not shown). The cosmid clone, c102D1 mapped immediately telomeric to LCR22-5 (Fig. 4A) in the immunoglobulin locus (22) and this probe was deleted from both the der(14) and the der(22), suggesting that the interval had been deleted (Fig. 4). The breakpoints for the balanced t(15;22) (BM798) and the balanced t(16;22) (GM02324) patients were telomeric to probe KB63E7 and were therefore distal to the LCR22s (BM798; Fig. 1; see below).

Table 2. Summary of breakpoint analysis. The cell line identifier as given by Coriell Cell Repository (GM numbers; http://arginine.umdnj.edu/index.html) or Morrow Lab (BM numbers) are listed in column one. The translocation for each cell line is listed in column two. The third column identifies the FISH probe tested proximal to the translocation breakpoint. The fourth column identifies the FISH probe tested distal to the breakpoint. The fifth column identifies at what LCR, if any, the breakpoint occurred. In the sixth column is listed the name of the probe used to identify the location of the translocation breakpoint on the partner chromosome. A check is made in the appropriate column, telomere unique or telomere distal, if the breakpoint occurred proximal to the most distally tested marker or distal to the most distally tested marker respectively.

Cell line	Translocation	22q11 proximal	22q11 distal	LCR22	Probe name	Telomere unique	Telomere distal
Balanced							
GM05878	t(10;22)	F5	BAC-361L10		Chromoprobe-T 10p and 10q	✓	
GM07511	t(14;22)	36N5	BAC-865E9	LCR22-5	dJ820M16/PAC	✓	
BM798	t(15;22)	KB63E7			124O5/PAC	✓	
BM819	t(16;22)	PAC-444P24	PAC-353B13	LCR22-3a		✓	
GM02324	t(16;22)	KB63E7			cGG4	✓	
GM00119	t(17;22)	PAC-444P24	PAC-353B13	LCR22-3a	95H6	✓	
GM03197	t(17;22)	CTA-677F7	PAC-493F17	LCR22-4	95H6	✓	
Unbalanced							
BM510	t(1;22)	PAC-444P24	PAC-353B13	LCR22-3a	14-e10/PAC		✓
GM03577	t(3;22)	PAC-201M18	PAC-353B13	LCR22-3a	196F4/PAC		✓
GM05401	t(4;22)	BAC-361L10	PAC-201M18		dJ963K6/PAC		✓
GM00980	t(11;22)	F5	BAC-361L10		dJ770G7/PAC		✓
GM06526	t(13;22)	CTD-2544O22	CTA-770H11		163-C9/PAC	✓	
BM58	t(18;22)	PAC-444P24	PAC-353B13	LCR22-3	52M11/P1		✓
GM11327	t(21;22)	PAC-901P22	CTD-2506I16	LCR22-4	PAC/63H24		✓

Unbalanced translocation lines that were monosomic for the 22pter–q11 regions

We performed similar FISH mapping studies on cell lines that contained unbalanced translocations resulting in partial monosomies for the 22pter–q11 region. The most centromeric translocation breakpoints occurred in cell line GM00980 (Table 1), between probes F5 and 361L10 (data not shown). One cell line, GM05401, had an unbalanced t(4;22) translocation with a breakpoint (Table 1) between probes 361L10 and 201M18 (25 kb centromeric to LCR22-3a; data not shown). Clone F5 contains the *HIRA* gene while clone 361L10 contains the *TBX1* gene and both map between LCR22-2 and LCR22-3a (Fig. 2A). Therefore, the breakpoints of GM05401 and GM00980 occurred in non-LCR22 sequences between LCR22-2 and LCR22-3a.

The three cell lines BM58, BM510 and GM03577 had unbalanced translocations and were monosomic for 22pter–q11. The breakpoints were placed between PAC 201M18/PAC444P24 and PAC353B13, placing the breakpoint in the immediate vicinity of LCR22-3a (Fig. 3).

The breakpoint in cell line GM06526 (Table 1) occurred in non-LCR22 sequences, in CTA770H10 (Fig. 2A), just centromeric to LCR22-3b (data not shown). The result was consistent with genotyping results, which placed the breakpoint between two genetic markers, D22S311 and D22S1709 (Fig. 1).

The translocation breakpoint on 22q11.2 in the unbalanced t(21;22) line, GM11327, occurred in the region containing LCR22-4, as determined by FISH mapping with proximal probes PAC901P22 (29 kb centromeric to LCR22-4) and distal probe CTD2506I16 (Fig. 2A). We performed FISH with clone RP11-165F18 that is 11 kb centromeric to LCR22-4 in order to narrow the breakpoint further. This clone was present on the normal chromosome 22 but missing from the der(21) chromosome (data not shown). Clone CTD2506I16 overlaps with LCR22-4 by 20 kb. This 20 kb region is absent in the more telomeric LCR22s (LCR22-5 to 8). These distal LCRs are the only LCR22s present on the derivative chromosome. Thus, the breakpoint for GM06526 is between RP11-165F18 and CTD2506I16, within LCR22-4 (Fig. 4). This finding was consistent with genotyping results (Fig. 1).

Combining both balanced and unbalanced translocations, most of the breakpoints occurred in the proximal half of 22q11.2. Genotyping and FISH mapping results were consistent with clusters of chromosome breakage within LCR22s, primarily LCR22-3a (Table 2). This suggests that, for the majority, the translocations might be mediated by the architectural features of this interval and others occurred by as yet unidentified mechanisms.

Somatic hybrid analysis

We further examined the breakpoints in BM58, BM510 and BM819, three separate lines mapping to LCR22-3a by hamster–human somatic cell hybrid analysis (Fig. 5A). PCR with primers surrounding and within LCR22-3a (16) was used to narrow the breakpoint interval (Fig. 5A). Markers RANBP1 and 444P24T7 map to unique sequences, 204 and 168 kb centromeric to LCR22-3a, respectively. The two markers, PRODH and DGCR6L map to the sc11.1 locus, 8 and 6 kb from LCR22-3a, respectively (Fig. 5A). The sc11.1 locus is duplicated, and the proximal locus is present at the telomeric end of LCR22-2 while the distal locus is present at the centromeric end of LCR22-3a (Fig. 5). Thus, if a PCR product is not detected in the der(1), der(16) and der(18) chromosomes of BM510, BM819 and BM58, respectively, then both copies are missing, as was found (Fig. 5B). The marker 7663.1 maps to the telomeric end of clone PAC444P24 (AC007663) and maps 478 bp centromeric to LCR22-3a (Fig. 5A). This marker was missing from the der(1), der(16) and der(18) chromosomes (Fig. 5B), indicating that the breakpoints were within LCR22-3a. The cell line BM819 was a balanced t(16;22) translocation carrier and, as expected, all the markers were present to 7663-1 (Fig. 5B). The USP18 gene maps to LCR22-2 and genomic

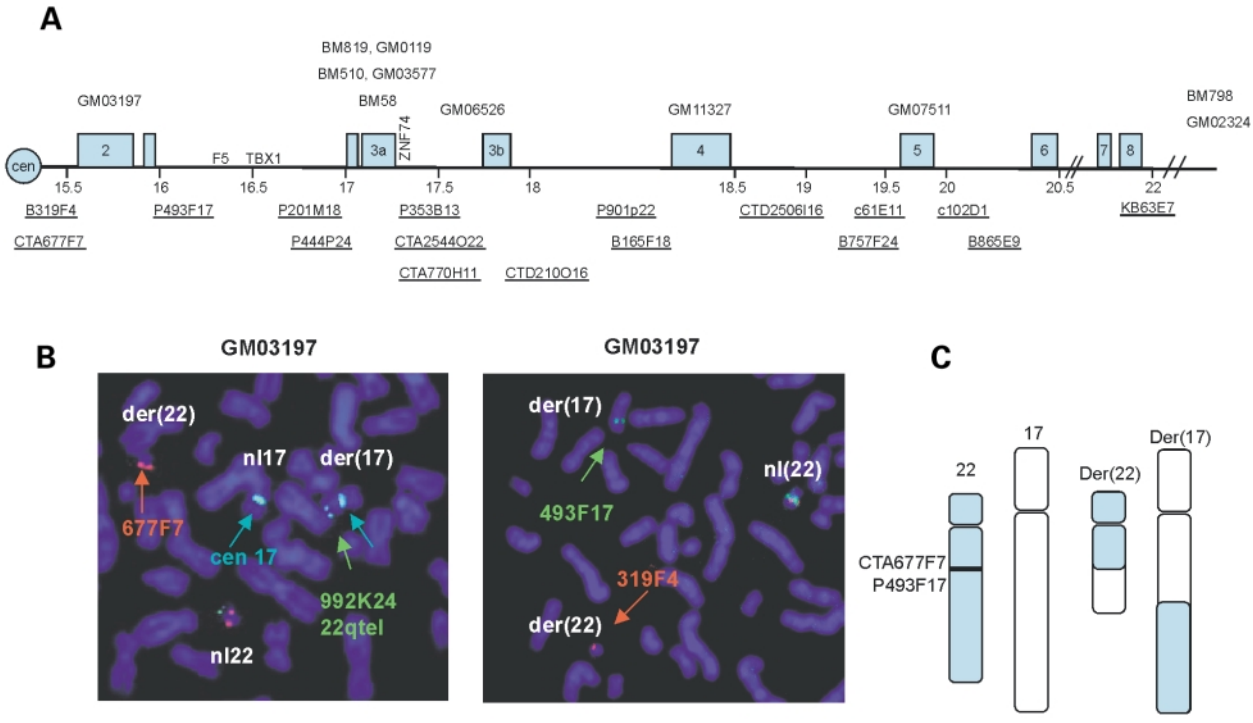


Figure 2. FISH mapping of chromosome 22q11.2 breakpoints. (**A**) The probes shown in the images are derived from clones listed below the line representing the 7.48 Mb chromosome 22q11.2 interval (LCR22 sizes shown in proportion to the interval). LCR22-2 maps to chr22: 15597234–15824819; LCR22-3a maps to chr22: 17249748–17433966; LCR22-3b maps to chr22: 17740485–17752123; LCR22-4 maps to chr22: 18164256–18409514; LCR22-5 maps to chr22: 19661949–19696275; LCR22-8 maps to chr22: 21700353–21790369 (Babcock *et al.*, manuscript in preparation; June 2002 assembly; http://genome.ucsc.edu/). The clones, BAC CTA-677F7 (AC008108; chr22: 15459502–15587142), PAC493F7 (proximal end is at chr22:15866430; clone maps telomeric to the sc11.1 locus) (14), PAC444P24 (AC007663; chr22: 17081510–17249748), PAC353B13 (ZNF74 maps to 5′ end of clone; chr22: 17450402), CTA-2544O22 (chr22: 17509522–17679048), CTD-210O16 (chr22: 17758515–17908679), BAC165F8 (chr22: 17987054–18152817), CTD-2506I16 (18388890–18594922) and BAC757F24 (chr22: 19481569–19665417) flank the LCR22s as shown. The map positions of cosmids 61E11 and 102D1 have been reported (22). The positions of the breakpoints in each cell line are shown as arrows. (**B**) FISH mapping of the cell line GM03197 (Table 1) with the probes shown in the LCR22 map of chromosome 22q11.2 (A) (Table 2). In addition, a probe from the centromeric region of chromosome 17 and the telomeric region of chromosome 22 (BAC 992K24) served as control. The positions of the breakpoints in each cell line are shown as arrows. The normal and derivative chromosomes are indicated in each image. The probe name is provided only once per image and it is color-coded with respect to the fluorescent label used to visualize the signal. The arrows in the images are also color-coded and depict each of two probes used. (**C**) The positions of the breakpoints on chromosomes 17 and 22 are illustrated. The probes on chromosome 22q11.2 that flank the breakpoint are shown.

clone AC008079. Exons within USP18 had become duplicated and transposed onto LCR22-3a (genomic clone AC007731) as shown in Figure 5A. If a PCR is performed using primers to amplify the unprocessed USP18 pseudogene, a product will be detected that derives from the functional gene and pseudogene. The functional USP18 gene is not present in the three derivative chromosomes of BM510, BM819 and BM58. The presence of a PCR product for USP18, in all the derivative chromosomes (Fig. 5B), indicates that the breakpoint occurred within LCR22-3a, centromeric to the unprocessed USP18 pseudogene (Fig. 5A). As expected, the next telomeric marker D22S264, located just telomeric to LCR22-3a was present the der(1), der(16) and der(18) chromosomes but missing from the der(22) chromosome in BM819 (Fig. 5A). The somatic cell hybrid results refine the FISH mapping studies and demonstrate that the breakpoints are within LCR22-3a.

Somatic hybrid analysis of BM798

The balanced carrier, BM798, had a breakpoint on chromosome 22 that was more telomeric to the LCR22s (Fig. 4). To determine the mechanism, we generated somatic hybrid cell lines. We performed a similar PCR analysis of different somatic hybrid cell lines containing both derivative chromosomes, the der(15) and the der(22) chromosomes (Fig. 6A). Chromosome 15 markers RH54439 to 68515-2 were present in the der(15) chromosome, while the next telomeric marker 68515-3 was absent but was present on the der(22) chromosome as were the more telomeric markers (Fig. 6A). Markers D22S1144 to 80241-2 were missing from the der(15) chromosome but were present on the der(22) chromosome, while the opposite was true for markers 80241-3 to NM_145862ex5 markers. Thus the breakpoint on chromosome 22 was in clone AL080241, mapping to 22q12.1, between markers 80241-2 and 80241-3, an interval of 498 bp (chr22: 25528524–25529022). The breakpoint on chromosome 22q12.1 was 836 kb centromeric to the Ewing Sarcoma breakpoint region (EWSR1; MIM 133450). The breakpoint on chromosome 15q26.2 was in a 361 bp interval (92581526–92581887). We then amplified the breakpoint junctions in both derivatives and subjected them to DNA sequence analysis. The sequences in the regions harboring the breakpoints on

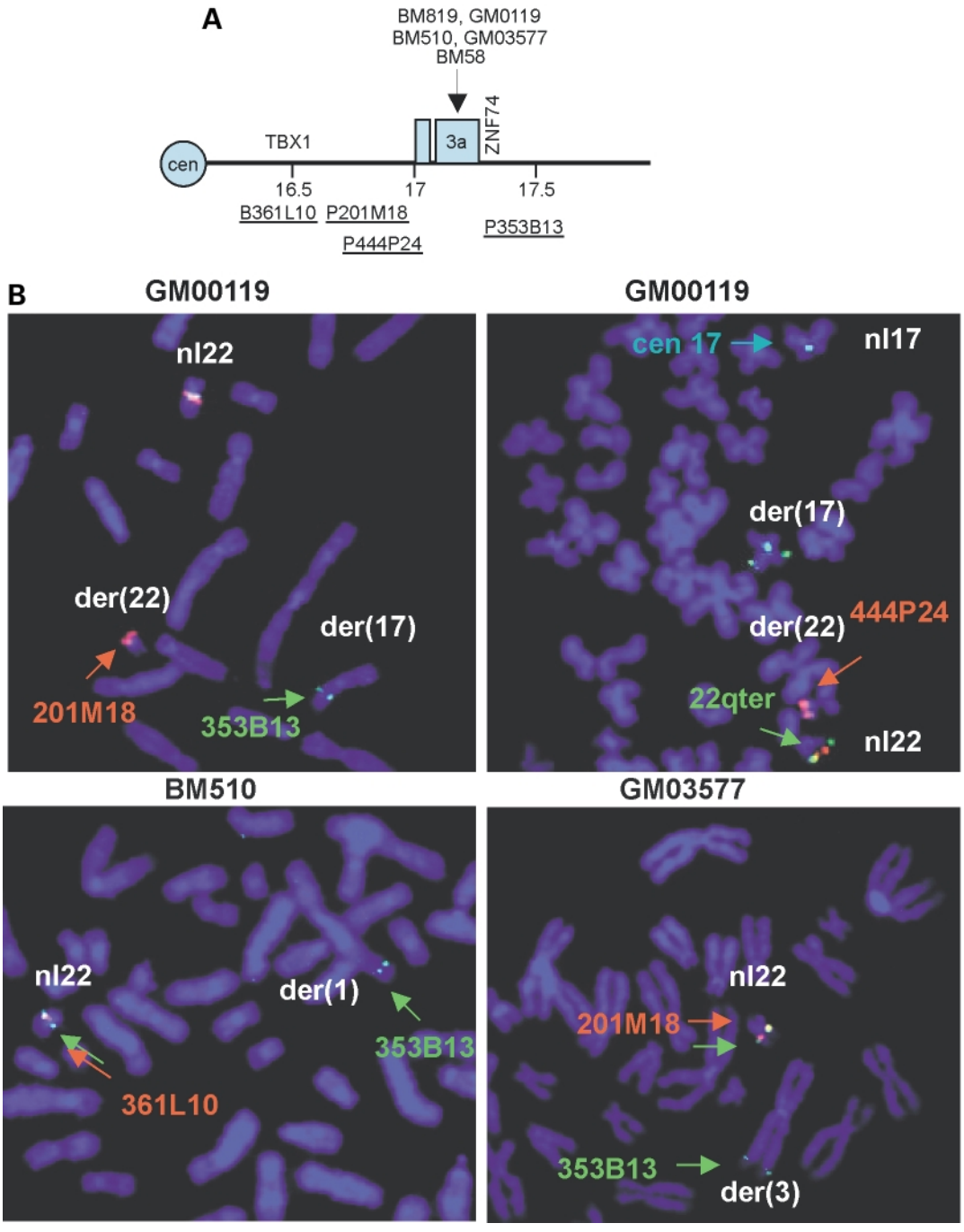


Figure 3. Definition of the breakpoints in LCR22-3a by FISH mapping. (**A**) The positions of the probes used for FISH mapping with respect to the LCR22-3a are indicated. (**B**) FISH mapping was performed with probes from genomic clones (Table 2) shown below the line representing chromosome 22q11.2. Representative images using the probes are shown for the cell lines, GM00119, BM510 and GM03577.

chromosome 15 and 22 were not similar, except for a short stretch of an AA on the wild-type chromosome 15 and an AAA on chromosome 22, leading to a deletion of the two-AA stretch on chromosome 15 in the der(22) chromosome (Fig. 6B). This suggests that the mechanism was probably random chromosome breakage and repair by non-homologous end joining.

Definition of the breakpoints on the partner chromosomes

To evaluate the region of chromosomal breakage in the telomeric band on the partner chromosomes, genetic markers were chosen based upon the reported karyotypes (D1S468, D1S2132 and D1S2633 for BM510 and D18S476, and D18S59

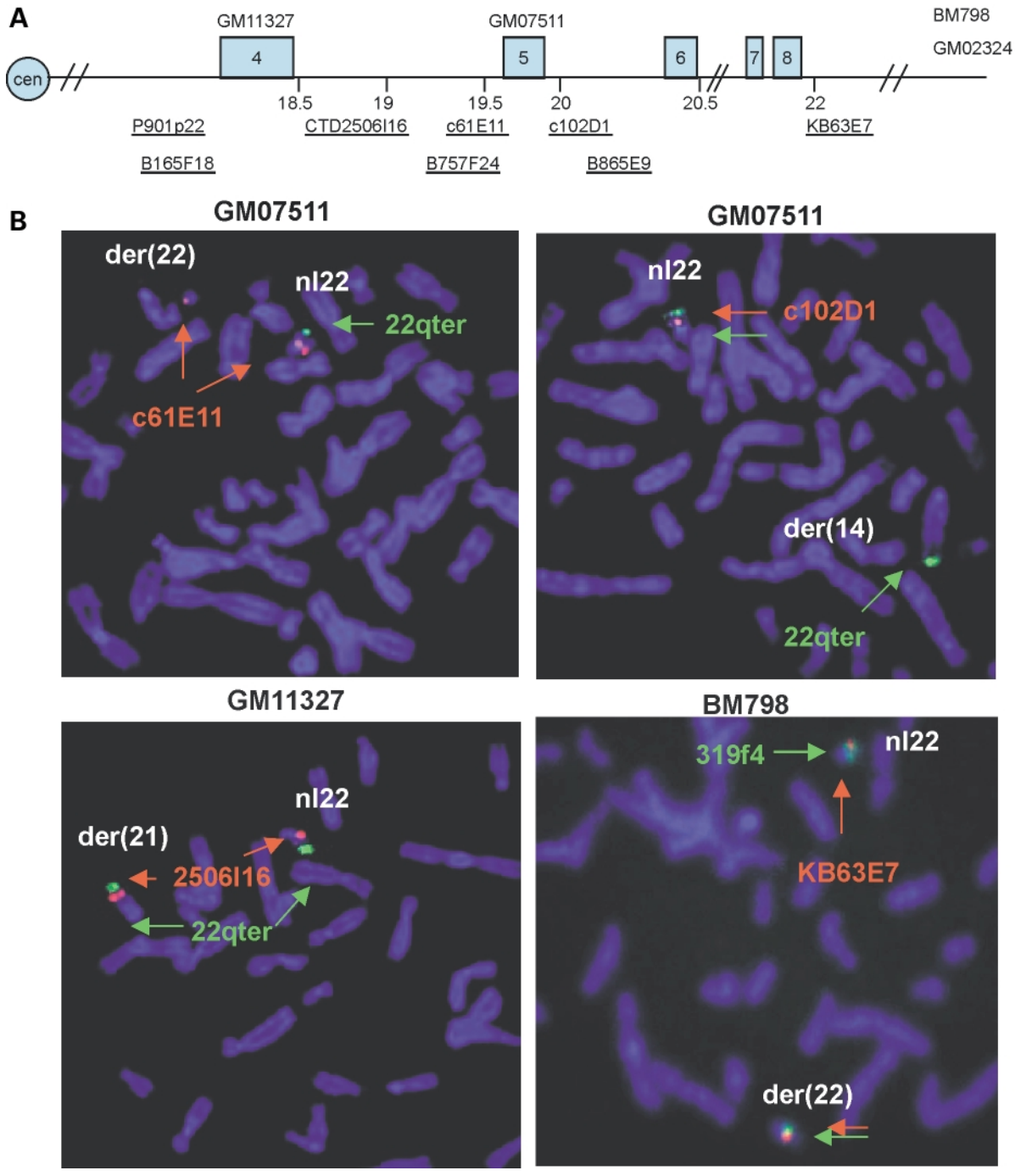


Figure 4. Examination of the breakpoints in cell lines mapping telomeric to LCR22-3a. (**A**) The positions of the probes used for FISH mapping with respect to the more distal LCR22s, are indicated. (**B**) FISH mapping was performed with probes from genomic clones (Table 2) shown below the line representing chromosome 22q11.2. Representative images using the probes are shown for the cell lines, GM07511, GM11327 and BM798.

for BM58). The breakpoint in BM819 on chromosome 16 occurred in a 903 kb interval (16q23.3; Chr16: 73237168–74140953), 3 Mb distal to the aphidicolin-inducible fragile site FRA16D gene, *WWOX* (NM_130792; Chr16: 69063544–70177366). We found that the breakpoints on both derivatives chromosome in BM58 and BM510 were telomeric to some of the last available genetic markers in the telomeric bands (D1S468, 1p36.32; chr1: 3023249–3023572; 3.3 Mb from the end; D18S59, 18p11.32; Chr18: 1253163–1253341). It has been estimated that the distance from the last available genetic marker on a chromosome to the end of the telomere is between 100 kb and 1 Mb (23). Therefore, the

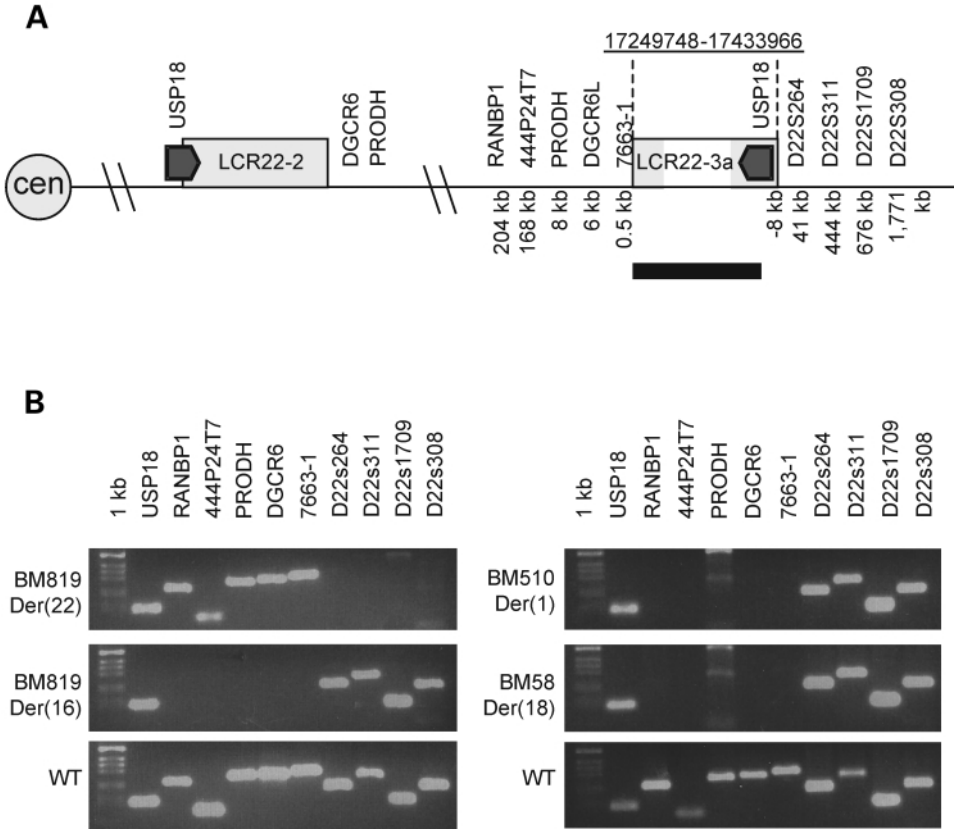


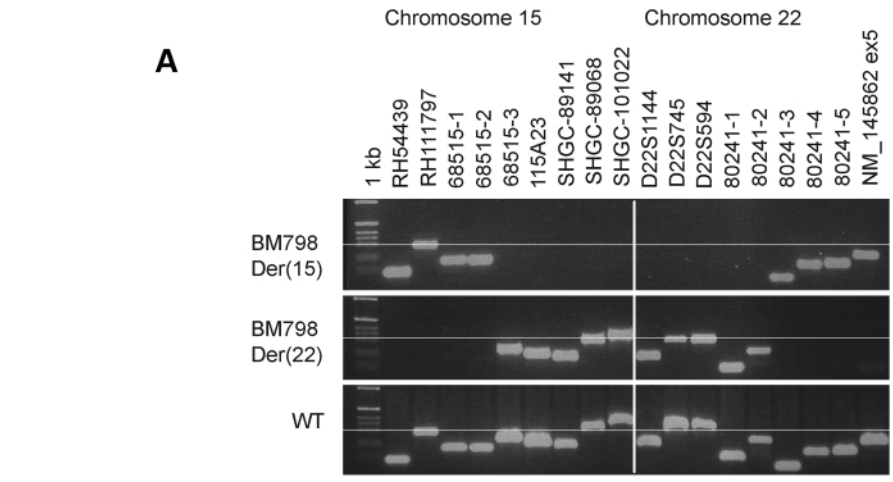
Figure 5. Somatic cell hybrid analysis of cell lines with breakpoints in LCR22-3a. (**A**) Individual PCR based markers were generated from the human genome sequence in the vicinity of LCR22-3a (the map coordinates are shown). The sc11.1 locus containing the DGCR6 and PRODH genes maps just telomeric to LCR22-2 and centromeric to LCR22-3a. The full-length USP18 gene (GenBank accession no. NM_017414) maps to LCR22-2. Internal exons from this gene have become duplicated and transposed to LCR22-3a, forming an unprocessed pseudogene, as indicated. The precise coordinates of the positions of the PCR markers are shown. The black bar indicates the interval containing the translocation breakpoint on 22q11.2. The separated wild-type and derivative chromosomes were examined in separate hamster–human somatic cell hybrid cell lines. (**B**) PCR mapping was performed to narrow the interval containing the translocation breakpoints on chromosome 22q11.2. The derivative chromosomes from the cell lines, BM819, BM510 and BM58 are shown. Of the three, only BM819 was a balanced translocation carrier, and therefore, has two derivative chromosomes (Table 1). The breakpoint on chromosome 22q11.2 occurred between the marker 7663-1 and USP18. The 1 kb ladder was used as a size control.

breakpoints occurred in two complex regions of the genome, the LCR22s and the most distal portion of the telomeric band of the partner chromosome. We cannot exclude the possibility that the translocation breakpoints occurred in the telomere associated repeats (TARs). After finding the translocation breakpoints in the last cytogenetic band of these two distinct chromosomes, we questioned if the breakpoint region on the partner chromosomes for the remaining translocations in this study also disrupted the last cytogenetic band. This prompted us to examine by FISH the region of chromosome breakage on all the partner chromosomes. It will be necessary to examine more cases harboring translocations in this interval to identify rearrangement hot-spots.

Characterization of partner chromosomes

To define the region of breakage in the partner chromosomes and to gain insight into possible mechanisms of translocation formation, we performed FISH mapping studies using probes for unique sequences in the telomeric bands of the partner chromosomes (Fig. 7). This allowed us to categorize the translocation breakpoints as centromeric or telomeric to the most distal known unique sequences on the partner chromosomes. Six cell lines had translocation breakpoints distal to the chromosome specific unique region probes (BM58, BM510, GM03577, GM05401, GM00980 and GM11327; Fig. 7). Eight cell lines (GM03197, GM05878, GM0119, GM06526, GM02324, GM07511, BM819 and BM798) were found to have breakpoints centromeric to the chromosome specific unique sequence probes (Fig. 7).

We examined the interval that was disrupted on the partner chromosome of GM02324 on 16p13 more closely because of the presence of two rearrangement disorders in this region, Rubinstein–Taybi syndrome (OMIM 180849) and alpha thalassemia. We found that the breakpoint for the balanced t(16;22) line, GM02324, was between the cyclic AMP-responsive element-binding protein (*CREBBP*) gene and the alpha globin gene cluster on chromosome 16 (Fig. 8). This is of interest because translocations in the intronic region and microdeletions in *CREBBP* are associated with Rubinstein–Taybi syndrome (24). Deletions of the end of



B

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Chr 15    GAGTTTCTACTTTGCTTTTTTAGTTGAGAC AATCACACTAGTAGGAAATATAGCATTAGTCATCTCTAATCTT
Der(15)   GAGTTTCTACTTTGCTTTTTTAGTTGAGAC AACTTAGTGAGCCATGTCCAGCTAGACTATGGAGTATTATC

Der(22)   GAGGAGAAGGCCCTCTAGAAAGGGGAAAGA TCACACTAGTAGGAAATATAGCATTAGTCATCTCTAATCTT
Chr 22    GAGGAGAAGGCCCTCTAGAAAGGGGAAAGA AACTTAGTGAGCCATGTCCAGCTAGACTATGGAGTATTATC
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Figure 6. Mapping and sequence analysis of the balanced t(15;22) translocation in BM798. (**A**) Hamster–human somatic hybrid cell lines were generated from lymphoblastoid cells from the individual, BM798. The two derivative chromosomes as well as a wild-type control were examined. PCR markers were generated that spanned the chromosomes 15 and 22 breakpoint intervals as shown (see supplement). The 1 kb ladder was used as a size control. (**B**) The breakpoint junctions on both derivative chromosomes were amplified by PCR with primers from markers that flank the chromosome breakpoints. The sequence that was nearest to the breakpoints is shown. The breakpoint on chromosome 15 was between the C at 92581643 and the A at 92581644 (line). The breakpoint on chromosome 22 was between the A at 25528665 and the A at 25528666 (line). The sequence was verified by examining the human genome sequence on the UCSC browser. Two As (bar) were deleted from the wildtype chromosome 15 on the der(22) chromosome.

the 16p arm can also remove the alpha globin genes, which are about 170–430 kb from the telomere (25), and may contribute to alpha thalassemia (26,27). Thus, this is a relatively unstable region in the genome. Notably it has also been suggested that the 22q11.2 region and the 16p13 region share ancestral origins due to paralogs mapping to the regions (28). A total of nine genes were found to have paralogs between chromosome 16p13 and 22q11.2 to 22q13 and, therefore, there is sequence similarity between these two regions (28).

Miller–Dieker syndrome (OMIM 247200) is caused by terminal deletions or malsegregation of translocations involving 17p13.3 (29,30). The translocation breakpoints in GM03197 and GM00119 were centromeric to the critical region associated with Miller–Dieker syndrome (MDS; Fig. 8). In addition, GM00119 was found to have a more complex rearrangement involving an insertion of chromatin containing the MDS region into chromosome 22, but retention of the 17p subtelomeric unique sequence probe on the derivative 17. Thus, genome architectural features in these intervals may be responsible for mediating the rearrangements.

DISCUSSION

Mechanism for 22q11.2 translocations

We investigated cell lines with unique 22q11.2 translocations to determine whether there were common intervals harboring chromosomal breakpoints. We found that most breakpoints occurred in the proximal half of chromosome 22q11.2, half in the LCR22s, and most of those in LCR22-3a. The 1.5 Mb VCFS/DGS distal deletion breakpoint and the constitutional t(11;22) translocation breakpoint occurs in this LCR22. Furthermore, two independent balanced t(17;22) translocations also occur in LCR22-3a (17,20,21). The breakpoints for the constitutional t(11;22) and t(17;22) are mediated by AT-rich palindromes on the partner chromosomes (17,19,21), implicating AT-rich palindromes in some of the cases.

Several other well-characterized genomic disorders involving deletions or duplications of at least 1 Mb are associated with their own region specific blocks of low copy repeats (LCRs) that lie just telomeric to the pericentromeric regions on selected chromosomes. These LCRs are thought to stimulate meiotic

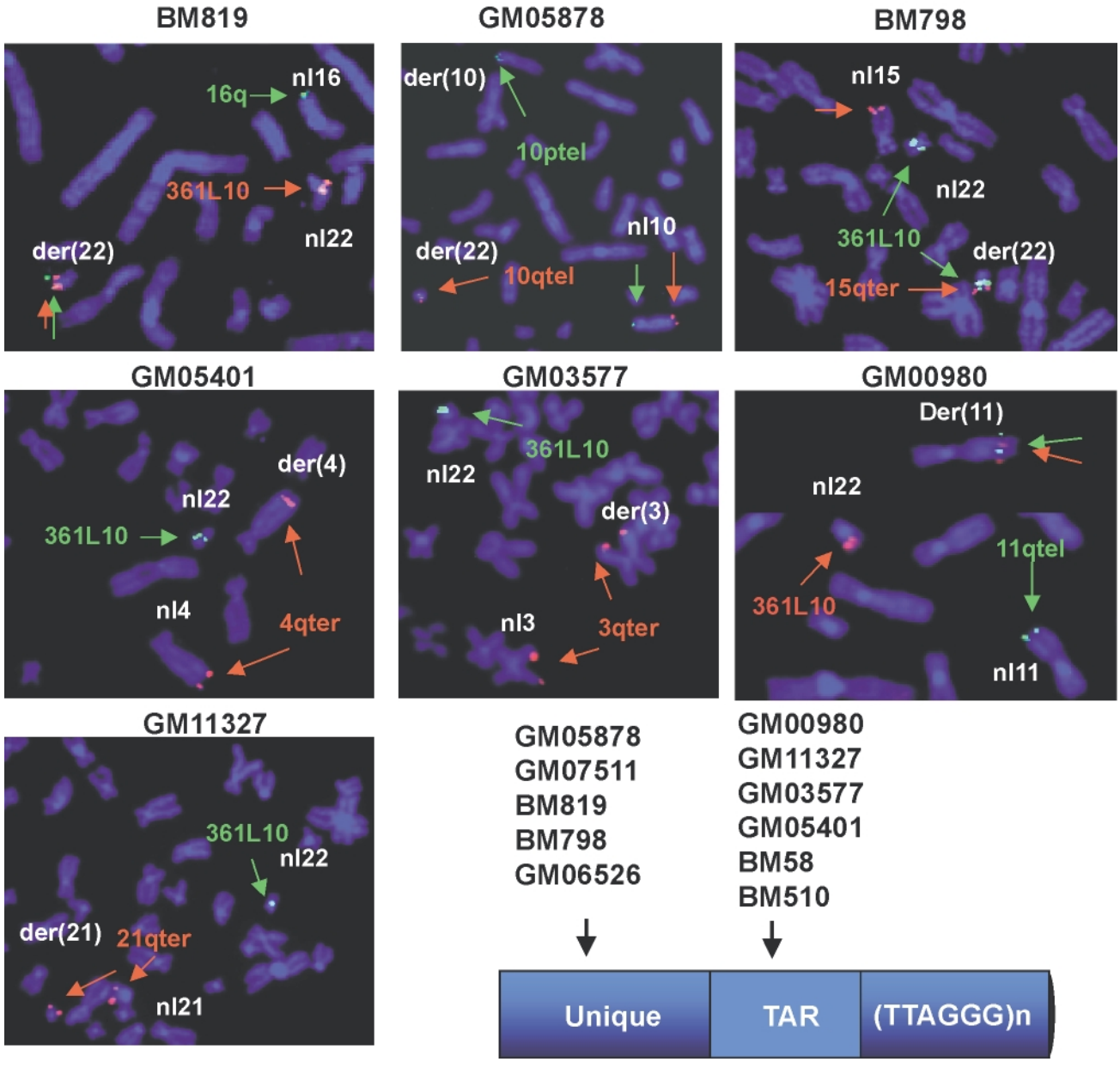

Figure 7. Map of translocation breakpoint regions within partner chromosomes by FISH mapping. FISH mapping was performed for all the cell lines in the study. Representative lines and images are shown. The map position of the probes are described (Table 2). The partner chromosome probes shown in the images derive from clones that are specific to the unique sequences in the telomeric bands of specific chromosomes (color-coded). Locations of breakpoints are indicated on an ideogram of a telomere region. Arrows in the images indicate the location of signals.

homologous recombination events. The best characterized genomic disorders on the autosomes, mediated by their own region specific LCRs, besides the ones on 22q11.2, include CMT1A/HNPP on chromosome 17p11–12 (Charcot–Marie–Tooth type 1A/hereditary neuropathy with pressure palsies; MIM 118220) and SMS (Smith–Magenis syndrome; MIM 182290) and its reciprocal duplication (31). In addition to these deletion/duplication disorders, non-recurrent chromosome rearrangements also occur on chromosome 17p, including translocations and unusually sized deletions (32). In a study of such rearrangements, deletion breakpoints were found in LCR sequences, some translocations occurred in centromeric alpha-satellite sequence, other translocations were within a highly repetitive pericentromeric segment, and one translocation was in the vicinity of an LCR (32). These data indicate that genomic architectural features may play important roles in mediating multiple types of chromosome rearrangements (32). In addition to the rearrangements on chromosome 17p, other recurrent rearrangements are mediated by LCRs including WBS (Williams–Beuren Syndrome; MIM 194050, chromosome 7q11.2), PWS/AS (Prader–Willi syndrome/Angelman syndrome; MIM 176270/105830, chromosome 15q11–q13)

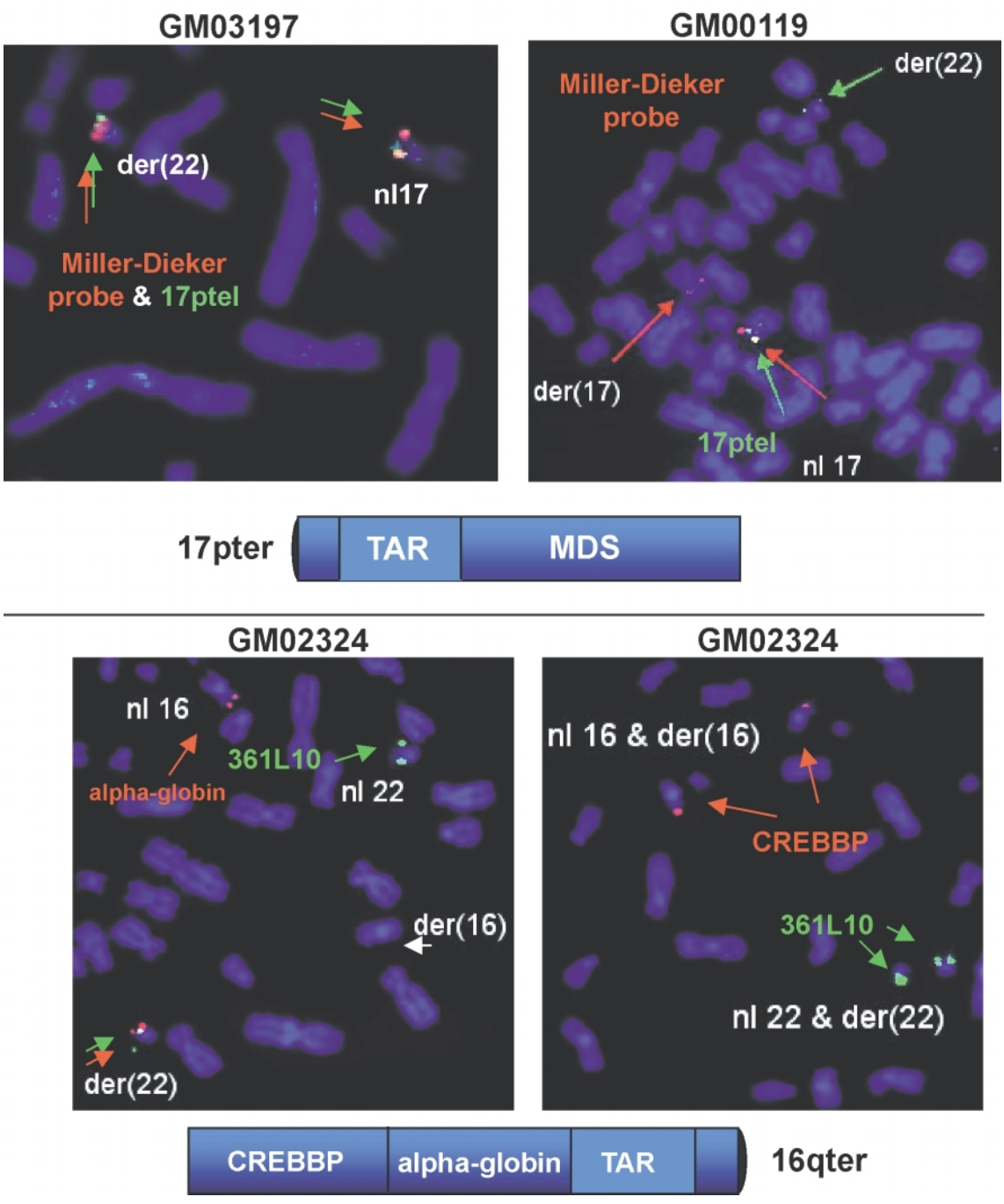

Figure 8. Cell lines with translocations in partner chromosomes in regions associated with genetic disorders. An ideogram of 17pter is shown with the position of the breakpoints for individuals GM03197 and GM00119. Both GM03197 and GM00119 have breakpoints that are centromeric to the Miller–Dieker syndrome (MDS) interval. The position of the translocation in GM02324 is shown and it is between *CREBBP* and *alpha globin* as illustrated in the ideogram under the FISH images.

and NF1 (neurofibromatosis type 1; chromosome 17q11.2) (reviewed in 33,34).

Mechanism for 22q11.2 and telomeric translocations

Studies examining the results of amniocenteses have shown that one in 2000 individuals are carriers for a *de novo* resciprocal translocation (35). It is of great interest to understand mechanisms leading to meiotic reciprocal translocations due to its impact on human health, infertility and fetal death. We found that most of the translocation breakpoints occurred near LCR22s and, as determined by G-banding and in many cases FISH analysis, all occurred in the most telomeric bands of partner chromosomes. Approximately half of the

breakpoints occurred distal to our most telomeric unique sequence markers. By combining the results of our study with that in the literature (46 cases total from the literature plus 14 in this report) (20,35–66), we found that 57% of 22q11.2 translocations involved the most terminal bands of random partner chromosomes. In the largest study of amniocentesis cases carrying reciprocal translocations (200 cases), 39% had one breakpoint in the most telomeric band (35). Based on the numbers ascertained for the 22q11.2 region and telomeres, combined with the epidemiological study, we suggest that there may be an increased frequency of rearrangements involving telomeric bands with 22q11.2, rather than a random distribution over all chromosome bands. There is a possibility of ascertainment bias as those with 22q11.2 deletions are viable and monosomy of a small segment of the very telomeric ends of chromosomes might be better tolerated than more proximal breaks that would results in greater imbalance. Nonetheless, those chromosomes involved would be subject to rearrangements by specific mechanisms such as that for LCR22-3a rearrangements and are worthy of further analysis. In addition, the possibility exists that in some of the cell lines additional rearrangements have occurred due to the process of creating a cell line and number of passages. This is unlikely to cause an overall alteration in the results but could have affected a small number of samples.

The dynamic aspect of the LCR22s has been illustrated in the multitude of recurrent rearrangements associated with 22q11.2. The precise mechanism for the different non-recurrent rearrangements as mediated by the LCR22s is not yet determined, but it is clear that they are implicated in disease and are therefore of significant clinical value. The dynamic aspect of LCR22s is also illustrated in a recent FISH mapping study in which LCR22 sequences were shown to occur on additional chromosomes besides 22q11.2, suggesting that they have expanded during primate evolution (67). Previous reports have noted a higher rate of telomeric rearrangements than rearrangements of other chromosomal segments (41,68). The telomeric regions are very dynamic and have shuffled during evolution between non-homologous chromosomes (69). Furthermore, the last 20 Mb present on chromosomes has been found to have higher rates of recombination (70), perhaps due to the presence of repeat sequences. Although there may be ascertainment bias towards imbalance of smaller segments as opposed to larger segments, one possible explanation for the increased incidence of 22q11.2 and telomere rearrangements, as compared to 22q11.2 and non-telomeric band rearrangements is the fact that two susceptible regions probably misalign and pair following chromosome breakage.

In some of the cell lines described in this report, the breakpoints occurred near the LCR22s but not within them. Chromosome rearrangements harboring breakpoint junctions within the vicinity but not precisely within LCRs have been identified. In fact, LCRs on chromosome 8p, comprising the olfactory receptor gene superfamily, has been shown to be responsible for chromosome rearrangements including inversions and translocations [inv dup(8p); t(4;8)(p16;p23) (71,72)]. Interestingly, the rearrangement breakpoints in patients with inversion duplications of 8p have breakpoints inside the LCRs but outside the actual repeat unit (71). This suggests that the breakpoint junction, or site of strand resolution, might not be precisely within the LCRs. This situation has been proposed for the t(9;22) BCR/ABL translocation associated with human leukemias (73). Interestingly, the BCR gene is present within LCR22-6 (15). A stretch of homology of 76 kb from the present 1400 kb centromeric to the ABL gene and 150 kb telomeric to the BCR gene (73). Thus, LCRs could map quite a distance from the actual chromosome breakpoint junctions, but they may mediate chromosome misalignments resulting in translocations.

We did not find LCR22 sequences in the vicinity of the breakpoints on the partner chromosomes by low stringency FISH mapping and by analysis of the human genome sequence. However, it is possible that the LCR22s themselves might provide genome instability to the interval by undefined mechanisms. It is also possible that smaller stretches of homology mediate the rearrangements. All the sequence is not available for all the telomeric regions and therefore it is not yet known whether subsets of LCR22 sequences will be elucidated in telomeric bands. The study of the 22q11.2 and telomeric translocations offers an important model for understanding how certain regions of the genome are susceptible to rearrangements, the mechanisms by which they occur, and how broken chromosomes are stabilized.

MATERIALS AND METHODS

Patients

Individuals possessing translocations with 'BM' codes (BM798, BM58, BM510 and BM819) were ascertained under an internal review board-approved program (CCI 99-201) and, for most, diagnosed as described elsewhere (16). Cell lines GM00980 (74–76), GM06526, GM03577, GM05401 (76,77), GM11327, GM00119, GM03197, GM05878 (74–76), GM02324 and GM07511 were purchased from the National Institute of General Medical Science cell repository (Coriell Cell Repositories NIGMS; http://locus.umdnj.edu/nigms/). The karyotypes and clinical features of each patient are listed in Table 1.

Somatic cell-hybrid cell lines

The methods used to generate hamster–human somatic hybrid cell lines from the translocation patients BM58, BM510, BM798 and BM819 have been described elsewhere (4). Individual clones were tested by PCR for retention of chromosomes 1, 15, 16, 18 and 22 with genetic markers. We are aware that subsequent rearrangements might have occurred in the somatic hybrids as compared with the parent blood cells, thus we have tested them with multiple PCR-based markers for verification. Markers for chromosome 1 were D1S179, D1S2891 and D1S2132; markers from chromosome 18 were D18S59, D18S565, D18S65, D18S384 and D18S476; markers from chromosome 15 were D15S657, D15S822, D15S642 and D15S659; markers from chromosome 16 were D16S475, D16S621, D16S671, D16S676, D16S688 and D16S771; and markers from chromosome 22 were D22S1709, D22S425, D22S1638, D22S686, D22S1638, D22S941, D22S689, D22S686, D22S444, D22S427 and D22S420. Two independent cell lines

were generated containing each derivative chromosome and all were analyzed in duplicate. The positive clones containing chromosome 1 (BM510), 15 (BM798), 16 (BM819), 18 (BM58) or 22 were expanded and genotyped using 20 ng template DNA with genetic markers spanning the chromosome 1p, 15q, 16q or 18p, and 22q11.2 regions, to confirm the integrity of the chromosomes and identity of the clones. Genotyping was also necessary to differentiate the content of the clones since the regions of breakage on the partner chromosomes in BM58 and BM510 were telomeric to any known genetic markers. PCR was performed on BM798 using the following primers: RH54439, RH111797, 68515-1, 68515-2, 68515-3, 115A23, SHGC-89141, SHGC-89068, SHGC-101022, D22s1144, D22s745, D22S594, 80241-1, 80241-2, 80241-3, 80241-4, 80241-5 and NM_145862 ex5. PCR was performed on BM819, BM58 and BM510 using the following primers: USP18, RanBP1, 444P24T7, PRODH, DGCR6, 7663-1, D22S264, D22S311, D22S1709 and D22S308.

FISH mapping

The clones used as probes for FISH mapping on chromosome 22 are shown in Figure 1 and listed in Table 2. Some of the clones have been previously described and they include, PAC 286O7 (4), BAC 361L10 (78), PAC 353B13 (16), PAC 901P22 (14), PAC 493F17 (14), F5 (79). The cosmids 48C12 (16) and 102D1 (80) were obtained from the Roswell Park Cancer Institute (LL22NC03 library). Clones KB113H7, KB318B8, KB1896H10 and KB63E7 were from the Keio BAC library, which are referred to as AP000347, AP000353, AP000355 and AP000359, respectively (81). BACs 165F18, 444p24, 361L10 and 757F24 were from the RPCI-11 BAC library (BACPAC Resource Center, Department of Cancer Genetics, Roswell Park Cancer Institute). The BACs 677F7 and 770H11 were obtained from the CITB-978SK CALTECH A BAC library. BACs 2506I16 and 2544O22 were obtained from the CITB-978SK CALTECH D BAC library. Probes used were PAC 196f4 (3qter, 3q29, GM03197), PAC dJ963k6 (4qter, 4q35, GM05401), Chromoprobe-T 10p and 10q (GM05878), PAC dJ770G7 (11qter, 11q25, GM00980), PAC163-C9 (13qter, 13q34, GM06526), PAC dJ820M16 (14qter, 14q32.3, GM07511), PAC 124O5 (15qter, 15q26.3, BM798), PAC 121-I4 (16pter, 16p13.3, GM02324), PAC 202L17 (17pter, 17p13.3, GM03197 and GM00119), PAC 63H24 (21qter, 21q22.3, GM11327), PAC 14-e10 (1p36, 1pter, BM510) and PAC 52M11 (18pter, BM58; Cytocell Ltd, UK). Additional probes on the partner chromosome used were cosmid RT100 for the Rubenstein–Taybi region (GM02324) (82), cGG4 (a-thalassemia, GM02324), 95H6 (GM03197 and GM00119) (83).

In one laboratory (AECOM), probes were labeled by nick translation at 15°C for 2 h. Repetitive sequence of the probes was blocked with a pre-hybridization at 37°C for 15 min with 100 µg of COT-1 DNA. Hybridization was overnight at 37°C. Biotinylated probes were detected with avidin-CY5, digoxygenin-labeled probes were detected with anti-digoxigenin-FITC, and DNP-labeled probes were detected with FITC-labeled (rabbit anti-sheep) anti-DNP antibodies. Chromosomes were stained with DAPI and were analyzed by fluorescence microscopy. In the other laboratory (BCM), 2-color FISH was performed as previously described (84).

ACKNOWLEDGEMENTS

We would like to thank Rosalie Goldberg and Merry Ferre for help in patient collection. We appreciate the efforts of Margaret Fusina in generating the BM798 somatic cell hybrid cell lines. We thank members of the California Institute of Technology and Bruce Roe at the University of Oklahoma for supplying us with BAC clones. We are grateful to family members who participated in this study. This work is supported by the MOD (1-FY00-768; B.E.M.) and NIH (1 PO-1 HD 39420-01; B.E.M. and L.G.S.; and 5 P01 HD34980-05, B.E.M.). E.S. is supported by a NIH training grant (GM 07491). T.S., S.M. and N.S. are supported from the 'Research for the Future' Program from the Japan Society for the Promotion of Science.

REFERENCES

1. Botto, L.D., Campbell, R.M., Coleman, K., Correa, A., Elixson, E.M., Erickson, J.D., Fernhoff, P., May, K., Merritt, R., O'Leary, L. *et al.* (2001) A population-based study of the 22q11.2 deletion. *Am. J. Hum. Genet.*, **69**, 583.
2. Burn and Goodship (1996) Congenital heart disease. In Rimoin, D.L., and Pyeritz, R.E. (eds), *Emory's and Rimoin's Principles and Practice of Medical Genetics*, 3rd edn. Churchill Livingstone, New York, Vol. 1, pp. 767–828.
3. Morrow, B., Goldberg, R., Carlson, C., Das Gupta, R., Sirotkin, H., Collins, J., Dunham, I., O'Donnell, H., Scambler, P., Shprintzen, R. *et al.* (1995) Molecular definition of the 22q11 deletions in velo-cardio-facial syndrome. *Am. J. Hum. Genet.*, **56**, 1391–1403.
4. Carlson, C., Sirotkin, H., Pandita, R., Goldberg, R., McKie, J., Wadey, R., Patanjali, S.R., Weissman, S.M., Anyane-Yeboa, K., Warburton, D. *et al.* (1997) Molecular definition of 22q11 deletions in 151 velo-cardio-facial syndrome patients. *Am. J. Hum. Genet.*, **61**, 620–629.
5. Budarf, M.L., Collins, J., Gong, W., Roe, B., Wang, Z., Bailey, L.C., Sellinger, B., Michaud, D., Driscoll, D.A. and Emanuel, B.S. (1995) Cloning a balanced translocation associated with DiGeorge syndrome and identification of a disrupted candidate gene. *Nat. Genet.*, **10**, 269–278.
6. Shaikh, T.H., Kurahashi, H., Saitta, S.C., O'Hare, A.M., Hu, P., Roe, B.A., Driscoll, D.A., McDonald-McGinn, D.M., Zackai, E.H., Budarf, M.L. *et al.* (2000) Chromosome 22-specific low copy repeats and the 22q11.2 deletion syndrome: genomic organization and deletion endpoint analysis. *Hum. Mol. Genet.*, **9**, 489–501.
7. Levy, A., Demczuk, S., Aurias, A., Depetris, D., Mattei, M.G. and Philip, N. (1995) Interstitial 22q11 microdeletion excluding the ADU breakpoint in a patient with DiGeorge syndrome. *Hum. Mol. Genet.*, **4**, 2417–2419.
8. 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.
9. 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**, 659–666.
10. Yamagishi, H., Garg, V., Matsuoka, R., Thomas, T. and Srivastava, D. (1999) A molecular pathway revealing a genetic basis for human cardiac and craniofacial defects. *Science*, **283**, 1158–1161.
11. McQuade, L., Christodoulou, J., Budarf, M., Sachdev, R., Wilson, M., Emanuel, B. and Colley, A. (1999) Patient with a 22q11.2 deletion with no overlap of the minimal DiGeorge syndrome critical region (MDGCR). *Am. J. Med. Genet.*, **86**, 27–33.
12. Mears, A.J., Duncan, A.M., 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.
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. Edelmann, L., Pandita, R.K. and Morrow, B.E. (1999) Low-copy repeats mediate the common 3-Mb deletion in patients with velo-cardio-facial syndrome. *Am. J. Hum. Genet.*, **64**, 1076–1086.

15. Edelmann, L., Pandita, R.K., Spiteri, E., Funke, B., Goldberg, R., Palanisamy, N., Chaganti, R.S., Magenis, E., Shprintzen, R.J. and Morrow, B.E. (1999) A common molecular basis for rearrangement disorders on chromosome 22q11. *Hum. Mol. Genet.*, **8**, 1157–1167.
16. Funke, B., Edelmann, L., McCain, N., Pandita, R.K., 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.
17. Edelmann, L., Spiteri, E., Koren, K., Pulijaal, V., Bialer, M.G., Shanske, A., Goldberg, R. and Morrow, B.E. (2001) AT-rich palindromes mediate the constitutional t(11;22) translocation. *Am. J. Hum. Genet.*, **68**, 1–13.
18. Kurahashi, H. and Emanuel, B.S. (2001) Long AT-rich palindromes and the constitutional t(11;22) breakpoint. *Hum. Mol. Genet.*, **10**, 2605–2617.
19. Kurahashi, H., Shaikh, T.H., Hu, P., Roe, B.A., Emanuel, B.S. and Budarf, M.L. (2000) Regions of genomic instability on 22q11 and 11q23 as the etiology for the recurrent constitutional t(11;22). *Hum. Mol. Genet.*, **9**, 1665–1670.
20. Kehrer-Sawatzki, H., Haussler, J., Krone, W., Bode, H., Jenne, D.E., Mehnert, K.U., Tummers, U. and Assum, G. (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.
21. Kurahashi, H., Shaikh, T., Takata, M., Toda, T. and Emanuel, B.S. (2003) The constitutional t(17;22): another translocation mediated by palindromic AT-rich repeats. *Am. J. Hum. Genet.*, **72**, 733–738.
22. Desmaze, C., Prieur, M., Amblard, F., Aikem, M., LeDeist, F., Demczuk, S., Zucman, J., Plougastel, B., Delattre, O., Croquette, M.F. *et al.* (1993) Physical mapping by FISH of the DiGeorge critical region (DGCR): involvement of the region in familial cases. *Am. J. Hum. Genet.*, **53**, 1239–1249.
23. Lese, C.M., Fantes, J.A., Riethman, H.C. and Ledbetter, D.H. (1999) Characterization of physical gap sizes at human telomeres. *Genome Res.*, **9**, 888–894.
24. Petrij, F., Giles, R.H., Dauwerse, H.G., Saris, J.J., Hennekam, R.C., Masuno, M., Tommerup, N., van Ommen, G.J., Goodman, R.H., Peters, D.J. *et al.* (1995) Rubinstein–Taybi syndrome caused by mutations in the transcriptional co-activator CBP. *Nature*, **376**, 348–351.
25. Wilkie, A.O., Higgs, D.R., Rack, K.A., Buckle, V.J., Spurr, N.K., Fischel-Ghodsian, N., Ceccherini, I., Brown, W.R. and Harris, P.C. (1991) Stable length polymorphism of up to 260 kb at the tip of the short arm of human chromosome 16. *Cell*, **64**, 595–606.
26. Wilkie, A.O., Lamb, J., Harris, P.C., Finney, R.D. and Higgs, D.R. (1990) A truncated human chromosome 16 associated with alpha thalassaemia is stabilized by addition of telomeric repeat (TTAGGG)n. *Nature*, **346**, 868–871.
27. Lamb, J., Harris, P.C., Wilkie, A.O., Wood, W.G., Dauwerse, J.G. and Higgs, D.R. (1993) De novo truncation of chromosome 16p and healing with (TTAGGG)n in the alpha-thalassemia/mental retardation syndrome (ATR-16). *Am. J. Hum. Genet.*, **52**, 668–676.
28. Giles, R.H., Dauwerse, H.G., van Ommen, G.J. and Breuning, M.H. (1998) Do human chromosomal bands 16p13 and 22q11-13 share ancestral origins? *Am. J. Hum. Genet.*, **63**, 1240–1242.
29. Schwartz, C.E., Johnson, J.P., Holycross, B., Mandeville, T.M., Sears, T.S., Graul, E.A., Carey, J.C., Schroer, R.J., Phelan, M.C., Szollar, J. *et al.* (1988) Detection of submicroscopic deletions in band 17p13 in patients with the Miller-Dieker syndrome. *Am. J. Hum. Genet.*, **43**, 597–604.
30. vanTuinen, P., Dobyns, W.B., Rich, D.C., Summers, K.M., Robinson, T.J., Nakamura, Y. and Ledbetter, D.H. (1988) Molecular detection of microscopic and submicroscopic deletions associated with Miller-Dieker syndrome. *Am. J. Hum. Genet.*, **43**, 587–596.
31. Potocki, L., Chen, K.S., Park, S.S., Osterholm, D.E., Withers, M.A., Kimonis, V., Summers, A.M., Meschino, W.S., Anyane-Yeboa, K., Kashork, C.D., Shaffer, L.G. and Lupski, J.R. (2000) Molecular mechanism for duplication 17p11.2- the homologous recombination reciprocal of the Smith–Magenis microdeletion. *Nat Genet.*, **24**, 84–87.
32. Stankiewicz, P., Shaw, C.J., Dapper, J.D., Wakui, K., Shaffer, L.G., Withers, M., Elizondo, L., Park, S.S. and Lupski, J.R., (2003) Genome architecture catalyzes nonrecurrent chromosomal rearrangements. *Am. J. Hum. Genet.*, **72**, 1101–1116.
33. 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.
34. Ji, Y., Eichler, E.E., Schwartz, S. and Nicholls, R.D. (2000) Structure of chromosomal duplicons and their role in mediating human genomic disorders. *Genome Res.*, **10**, 597–610.
35. Warburton, D. (1991) *De novo* balanced chromosome rearrangements and extra marker chromosomes identified at prenatal diagnosis: clinical significance and distribution of breakpoints. *Am. J. Hum. Genet.*, **49**, 995–1013.
36. Back, E., Stier, R., Bohm, N., Adlung, A. and Hameister, H. (1980) Partial monosomy 22pter leads to q11 in a newborn with the clinical features of trisomy 13 syndrome. *Ann. Genet.*, **23**, 244–248.
37. Greenberg, F., Elder, F.F., Haffner, P., Northrup, H. and Ledbetter, D.H. (1988) Cytogenetic findings in a prospective series of patients with DiGeorge anomaly. *Am. J. Hum. Genet.*, **43**, 605–611.
38. Greenberg, F., Crowder, W.E., Paschall, V., Colon-Linares, J., Lubianski, B. and Ledbetter, D.H. (1984) Familial DiGeorge syndrome and associated partial monosomy of chromosome 22. *Hum. Genet.*, **65**, 317–319.
39. Kelley, R.I., Zackai, E.H., Emanuel, B.S., Kistenmacher, M., Greenberg, F. and Punnett, H.H. (1982) The association of the DiGeorge anomalad with partial monosomy of chromosome 22. *J. Pediatr.*, **101**, 197–200.
40. Nukina, S., Nishimura, Y., Kinugasa, A., Sawada, T., Hamaoka, K., Inazawa, J., Tsuda, S. and Abe, T. (1989) A case of incomplete DiGeorge syndrome associated with partial monosomy 22q11.1 due to maternal 14;22 translocation. *Jinrui Idengaku Zasshi*, **34**, 235–241.
41. Aurias, A., Prieur, M., Dutrillaux, B. and Lejeune, J. (1978) Systematic analysis of 95 reciprocal translocations of autosomes. *Hum. Genet.*, **45**, 259–282.
42. Stoll, C. (1980) Nonrandom distribution of exchange points in patients with reciprocal translocations. *Hum. Genet.*, **56**, 89–93.
43. Carratala, F., Galan, F., Moya, M., Estivill, X., Pritchard, M.A., Llevadot, R., Nadal, M. and Gratacos, M. (1998) A patient with autistic disorder and a 20/22 chromosomal translocation. *Dev. Med. Child Neurol.*, **40**, 492–495.
44. Rees, M.I., Worwood, M., Thompson, P.W., Gilbertson, C. and May, A. (1994) Red cell dimorphism in a young man with a constitutional chromosomal translocation t(11;22)(p15.5;q11.21). *Br. J. Haematol.*, **87**, 386–395.
45. Kerstjens-Frederikse, W.S., Kurahashi, H., Driscoll, D.A., Budarf, M.L., Emanuel, B.S., Beatty, B., Scheidl, T., Siegel-Bartelt, J., Henderson, K., Cytrynbaum, C. *et al.* (1999) Microdeletion 22q11.2: clinical data and deletion size. *J. Med. Genet.*, **36**, 721–723.
46. Lupski, J.R., Langston, C., Friedman, R., Ledbetter, D.H. and Greenberg, F. (1991) Di George anomaly associated with a *de novo* Y;22 translocation resulting in monosomy del(22)(q11.2). *Am. J. Med. Genet.*, **40**, 196–198.
47. Reddy, K.S., Sulcova, V. and Siassi, B. (1996) Two sibs with Wolf-Hirschhorn and DiGeorge deletions resulting from an unbalanced chromosome rearrangement, 45, XX/XY, der(4)t(4;22) (p16.3;q11.2) mat,-22. *J. Med. Genet.*, **33**, 852–855.
48. Causey, T., Abramson, R.K., Malone, T. and Best, R.G. (2001) Psychosis and ataxia in a patient with a novel 46, XX, t(1;22)(p36.3;q11.2) translocation. *Am. J. Hum. Genet.*, **69**, 562.
49. Li, M.M., Jackson, K. and Pridjian, G. (2001) A familial t(6;22) translocation resulting in both Duane syndrome and velo-cardio-facial syndrome in siblings. *Am. J. Hum. Genet.*, **69**, 688.
50. Krueger, A., Reichl, R., Stankiewicz, P. and Hansmann, I. (2001) Learning difficulties, minor dysmorphic features and interstitial telomeric sequences in a boy with 45, XY and a 5;22 translocation. *Am. J. Hum. Genet.*, **69**, 806.
51. de la Chapelle, A., Herva, R., Koivisto, M. and Aula, P. (1981) A deletion in chromosome 22 can cause DiGeorge syndrome. *Hum. Genet.*, **57**, 253–256.
52. Raymond, F.L., Bovie, C., Holden, S., Schroer, A. and Trump, D. (2001) Neurofibromatosis type 2 in a female patient with a balanced de novo X;22 translocation. *Am. J. Hum. Genet.*, **69**, 315.
53. Augusseau, S., Jouk, S., Jalbert, P. and Prieur, M. (1986) DiGeorge syndrome and 22q11 rearrangements. *Hum. Genet.*, **74**, 206.
54. 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.
55. Van Hove, J.L., McConkie-Rosell, A., Chen, Y.T., Iafolla, A.K., Lanman, J.T., Jr, Hennessy, M.D. and Kahler, S.G. (1992) Unbalanced translocation 46, XY, -15, +der(22)t(15;22)(q13;q11)pat: case report and review of the literature. *Am. J. Med. Genet.*, **44**, 24–30.

56. Schwanitz, G. and Zerres, K. (1987) Partial monosomy 22 as result of an X/22 translocation in a newborn with DiGeorge syndrome. *Ann. Genet.*, **30**, 80–84.
57. Fried, K., Goldberg, M.D. and Rosenblatt, M. (1980) Proximal 14 trisomy 46, XX, -22 +der(14)t(14;22) (q21;q11)mat. *Teratology*, **21**, 309–312.
58. Mutchinick, O., Ruz, L. and Jimenez, R. (1978) Partial trisomies 13 and 22 due to nondisjunction of a maternal reciprocal translocation, t(13;22)(q22;q11). *Hum. Genet.*, **45**, 89–95.
59. Pivnick, E.K., Wilroy, R.S., Summitt, J.B., Tucker, B., Herrod, H.G. and Tharapel, A.T. (1990) Adjacent-2 disjunction of a maternal t(9;22) leading to duplication 9pter–q22 and deficiency of 22pter–q11.2. *Am. J. Med. Genet.*, **37**, 92–96.
60. Jancar, J. and Karki, C.B. (1989) Unbalanced form of translocation deletion between chromosomes 6 and 22 in a mentally handicapped female [45, XX, -6, -22, +der(6),t(6;22)(q25.1:q11.2)]. *J. Ment. Defic. Res.*, **33**(Pt 3), 255–259.
61. Jaquez, M., Driscoll, D.A., Li, M., Emanuel, B.S., Hernandez, I., Jaquez, F., Lembert, N., Ramirez, J. and Matalon, R. (1997) Unbalanced 15;22 translocation in a patient with manifestations of DiGeorge and velocardiofacial syndrome. *Am. J. Med. Genet.*, **70**, 6–10.
62. Ledbetter, D.H., Rich, D.C., O'Connell, P., Leppert, M. and Carey, J.C. (1989) Precise localization of NF1 to 17q11.2 by balanced translocation. *Am. J. Hum. Genet.*, **44**, 20–24.
63. Faed, M.J., Robertson, J., Beck, J.S., Cater, J.I., Bose, B. and Madlom, M.M. (1987) Features of di George syndrome in a child with 45, XX, -3, -22, +der(3),t(3;22)(p25;q11). *J. Med. Genet.*, **24**, 225–227.
64. Brondum-Nielsen, K. (1991) Extra small marker chromosome associated with normal phenotype due to 3:1 disjunction of t(14;22) in a parent. Implications for the origin of marker chromosomes. *Clin. Genet.*, **40**, 215–217.
65. Cannizzaro, L.A., Aronson, M.M. and Emanuel, B.S. (1985) *In situ* hybridization and translocation breakpoint mapping. II. Two unusual t(21;22) translocations. *Cytogenet. Cell Genet.*, **39**, 173–178.
66. Moore, J.W., Hyman, S., Antonarakis, S.E., Mules, E.H. and Thomas, G.H. (1986) Familial isolated aniridia associated with a translocation involving chromosomes 11 and 22 [t(11;22)(p13;q12.2)]. *Hum. Genet.*, **72**, 297–302.
67. Bailey, J.A., Yavor, A.M., Viggiano, L., Misceo, D., Horvath, J.E., Archidiacono, N., Schwartz, S., Rocchi, M. and Eichler, E.E. (2002) Human-specific duplication and mosaic transcripts: the recent paralogous structure of chromosome 22. *Am. J. Hum. Genet.*, **70**, 83–100.
68. Jacobs, P.A., Buckton, K.E., Cunningham, C. and Newton, M. (1974) An analysis of the break points of structural rearrangements in man. *J. Med. Genet.*, **11**, 50–64.
69. Mefford, H.C. and Trask, B.J. (2002) The complex structure and dynamic evolution of human subtelomeres. *Nat. Rev. Genet.*, **3**, 91–102.
70. International Human Genome Sequencing Consortium (2001) Initial sequencing and analysis of the human genome. *Nature*, **409**, 860–921.
71. Giglio, S., Broman, K.W., Matsumoto, N., Calvari, V., Gimelli, G., Neumann, T., Ohashi, H., Voullaire, L., Larizza, D., Giorda, R., Weber, J.L., Ledbetter, D.H. and Zuffardi, O. (2001) Olfactory receptor-gene clusters, genomic-inversion polymorphisms, and common chromosome rearrangements. *Am. J. Hum. Genet.*, **68**, 874–883.
72. Giglio, Calvari, V., Gregato, G., Gimelli, G., Camanini, S., Giorda, R., Ragusa, A., Guerneri, S., Selicorni, A., Stumm, M., Tonnies, H., Ventura, M., Zollino, M., Neri, G., Barber, J., Wieczorek, D., Rocchi, M. and Zuffardi, O. (2002) Heterozygous submicroscopic inversions involving olfactory receptor–gene clusters mediate the recurrent t(4;8)(p16;p23) translocation. *Am. J. Hum. Genet.*, **71**, 276–285.
73. Saglio, G., Storlazzi, C.T., Giugliano, E., Surace, C., Anelli, L., Rege-Cambrin, G., Zagaria, A., Jimenez Velasco, A., Heiniger, A., Scaravaglio, P., Torres Gomez, A., Roman Gomez, J., Archidiacono, N., Banfi, S. and Rocchi, M. (2002) A 76-kb duplicon maps close to the BCR gene on chromosome 22 and the ABL gene on chromosome 9: possible involvement in the genesis of the Philadelphia chromosome translocation. *Proc. Natl Acad. Sci. USA*, **99**, 9882–9887.
74. Kurahashi, H., Akagi, K., Inazawa, J., Ohta, T., Niikawa, N., Kayatani, F., Sano, T., Okada, S. and Nishisho, I. (1995) Isolation and characterization of a novel gene deleted in DiGeorge syndrome. *Hum. Mol. Genet.*, **4**, 541–549.
75. Fu, W., Borgaonkar, D.S., Ladewig, P.P., Weaver, J. and Pomerance, H.H. (1976) Structural aberrations of the long arm of chromosome no. 22. Report of a family with translocation t(11;22) (q25;q11). *Clin. Genet.*, **10**, 329–336.
76. 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.
77. Cannizzaro, L.A. and Emanuel, B.S. (1985) *In situ* hybridization and translocation breakpoint mapping. III. DiGeorge syndrome with partial monosomy of chromosome 22. *Cytogenet. Cell Genet.*, **39**, 179–183.
78. Funke, B., Epstein, J.A., Kochilas, L.K., Lu, M.M., Pandita, R.K., Liao, J., Bauerndistel, R., Schuler, T., Schorle, H., Brown, M.C. *et al.* (2001) Mice overexpressing genes from the 22q11 region deleted in velo-cardio-facial syndrome/DiGeorge syndrome have middle and inner ear defects. *Hum. Mol. Genet.*, **10**, 2549–2556.
79. 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.
80. 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.
81. Dunham, I., Shimizu, N., Roe, B.A., Chissoe, S., Hunt, A.R., Collins, J.E., Bruskiewich, R., Beare, D.M., Clamp, M., Smink, L.J. *et al.* (1999) The DNA sequence of human chromosome 22. *Nature*, **402**, 489–495.
82. Breuning, M.H., Dauwerse, H.G., Fugazza, G., Saris, J.J., Spruit, L., Wijnen, H., Tommerup, N., van der Hagen, C.B., Imaizumi, K., Kuroki, Y. *et al.* (1993) Rubinstein–Taybi syndrome caused by submicroscopic deletions within 16p13.3. *Am. J. Hum. Genet.*, **52**, 249–254.
83. Pilz, D.T., Macha, M.E., Precht, K.S., Smith, A.C., Dobyns, W.B. and Ledbetter, D.H. (1998) Fluorescence in situ hybridization analysis with LIS1 specific probes reveals a high deletion mutation rate in isolated lissencephaly sequence. *Genet. Med.*, **1**, 29–33.
84. Shaffer, L.G., McCaskill, C., Han, J.Y., Choo, K.H., Cutillo, D.M., Donnenfeld, A.E., Weiss, L. and Van Dyke, D.L. (1994) Molecular characterization of de novo secondary trisomy 13. *Am. J. Hum. Genet.*, **55**, 968–974.