

Genome Architecture Catalyzes Nonrecurrent Chromosomal Rearrangements

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To investigate the potential involvement of genome architecture in nonrecurrent chromosome rearrangements, we analyzed the breakpoints of eight translocations and 18 unusual-sized deletions involving human proximal 17p. Surprisingly, we found that many deletion breakpoints occurred in low-copy repeats (LCRs); 13 were associated with novel large LCR17p structures, and 2 mapped within an LCR sequence (middle SMS-REP) within the Smith-Magenis syndrome (SMS) common deletion. Three translocation breakpoints involving 17p11 were found to be located within the centromeric α -satellite sequence D17Z1, three within a pericentromeric segment, and one at the distal SMS-REP. Remarkably, our analysis reveals that LCRs constitute >23% of the analyzed genome sequence in proximal 17p—an experimental observation two- to fourfold higher than predictions based on virtual analysis of the genome. Our data demonstrate that higher-order genomic architecture involving LCRs plays a significant role not only in recurrent chromosome rearrangements but also in translocations and unusual-sized deletions involving 17p.

Introduction

The molecular bases of recurrent interstitial chromosomal deletions and duplications have been uncovered only recently. Most of these rearrangements result from meiotic homologous recombination between nonallelic copies of low-copy repeats (LCRs). The involvement of genome architectural features in susceptibility to rearrangements resulting in disease traits appears to be a general phenomenon. These conditions have been referred to as “genomic disorders” (Lupski 1998, 2003). The number of recognized genomic disorders continues to rise (Emanuel and Shaikh 2001; Inoue and Lupski 2002; Stankiewicz and Lupski 2002a); simultaneously with the discovery, by computational analysis, that LCRs (>10 kb; >95%–97% identity) comprise 5%–10% of the human genomic sequence (Bailey et al. 2001). Thus, the human genome is laden with clusters of LCRs, most of which appear to have evolved during primate speciation

(Stankiewicz and Lupski 2002b), making it likely that genome rearrangements will continue to be recognized as playing a major role in human disease and potentially in the evolution of the human species.

We were interested in investigating the molecular basis of nonrecurrent chromosome rearrangements, such as translocations and unusual-sized deletions, that occurred in genomic regions in which recurrent rearrangements have been identified previously. To elucidate the molecular mechanisms, we analyzed extensively the rearrangement breakpoints. Because of a wealth of information with regard to the complete genome sequence (International Human Genome Sequencing Consortium [IHGSC] 2001; Venter et al. 2001), structure of LCRs, and knowledge of higher-order genome architecture (Bi et al. 2002; Inoue and Lupski 2002; Park et al. 2002; Stankiewicz and Lupski 2002a), we focused on breakpoints within proximal 17p in patients with nonrecurrent rearrangements.

The gene-rich and highly unstable human genomic region 17p11.2-p12 has been found to be rearranged in a variety of different structural chromosome aberrations. The same ~1.4-Mb genomic fragment within chromosome 17p12 is duplicated and deleted, respectively, in patients with Charcot-Marie-Tooth type 1A disease (CMT1A) and in patients with hereditary neuropathy with liability to pressure palsies (HNPP) (Chance et al. 1994; Reiter et al. 1996). This genomic segment is flanked by two ~24-kb LCRs, termed the “proximal CMT1A-REP” and the “distal CMT1A-REP” (fig. 1), which serve

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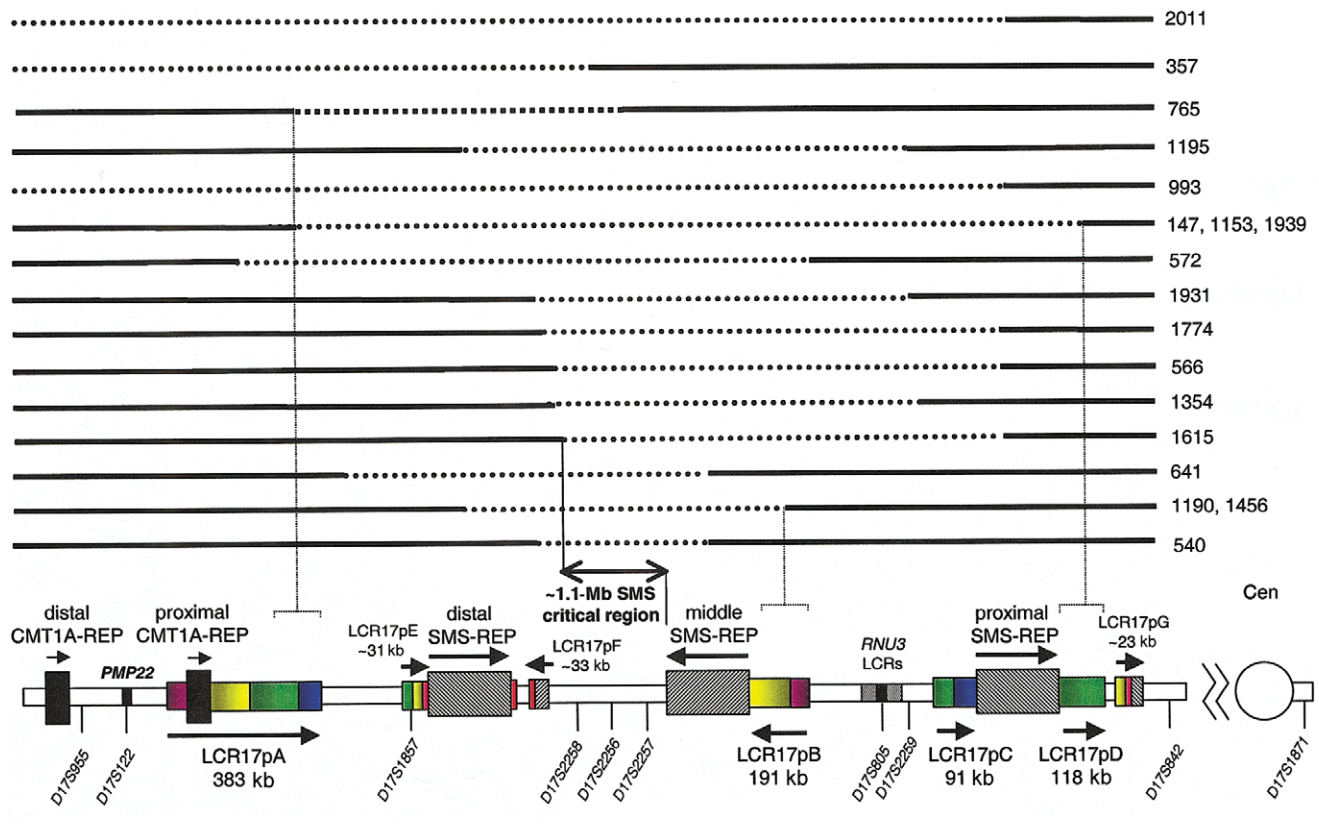


Figure 1 Breakpoint analysis of unusual-sized deletions. Proximal chromosome 17p is depicted at the bottom, showing the size, position, and orientation of LCRs. Dashed horizontal lines represent the genomic segment deleted for 18 different patients, and solid horizontal lines depict the retained genomic material, with the patient number shown to the right. The LCR17p structures are depicted in colors, to better represent their homology and orientation with respect to each other; the closed arrowheads represent the orientation of the LCR17p subunits. Selected breakpoints involving the LCR17ps are shown as vertical dashed lines. The horizontal line flanked by open arrowheads (below the genomic segments) depicts the SMS critical region; the common deletion (80%–90% of patients with SMS) occurs between proximal and distal SMS-REP copies. Note that the distal deletion breakpoints in patients 357, 993, and 2011 map outside the analyzed genomic region and thus were not included in the calculation of the percentage of chromosome breakpoints associated with LCRs in proximal 17p. Only >20-kb LCRs are depicted. The map is not to scale.

as substrates for nonallelic homologous recombination (NAHR) (Pentao et al. 1992; Reiter et al. 1997). The same LCRs/NAHR-based mechanism results in del(17)(p11.2p11.2), causing Smith-Magenis syndrome (SMS [MIM 182290]) and the newly described duplication dup(17)(p11.2p11.2) syndrome (Chen et al. 1997; Potocki et al. 2000). On the basis of the identification of recurrent junction fragments by pulsed-field gel electrophoresis (PFGE), these genomic disorders were shown to be caused by the reciprocal deletion and duplication, respectively, of the same ~4-Mb genomic region in chromosome 17p11.2 in 80%–90% of patients. The rearranged segment is flanked by the proximal SMS-REP (~256 kb) and the distal SMS-REP (~176 kb) LCRs (Chen et al. 1997; Potocki et al. 2000; Park et al. 2002); a third LCR copy, the middle SMS-REP (~241 kb), maps between them and is inverted in orientation (fig. 1) (Park et al. 2002).

In addition to the CMT1A-REP and SMS-REP LCRs,

we recently described a novel large LCR family termed “LCR17p” (Park et al. 2002). These LCRs are localized in 17p11.2-p12 around the proximal CMT1A-REP (~383-kb LCR17pA), adjacent to the middle SMS-REP on the centromeric side (~191-kb LCR17pB), and flanking the proximal SMS-REP (~91-kb LCR17pC and ~118-kb LCR17pD) (fig. 1). An ancestral genomic interval syntenic to the LCR17pA has been shown to be involved in the origin of an evolutionary chromosome translocation t(4;19) in *Gorilla gorilla* (Stankiewicz et al. 2001).

The majority of the chromosome aberrations reported (Brewer et al. 1998, 1999) appear to have random breakpoints, whereas, for recurrent interstitial deletions and reciprocal duplications, the breakpoints are associated with particular genomic architectural features (e.g., LCRs, AT-rich palindromes, and fragile sites) that mediate the recurrence of the aberrations (Lupski 1998; Shaffer and Lupski 2000; Emanuel and Shaikh

2001; Richards 2001; Inoue and Lupski 2002; Stankiewicz and Lupski 2002a). To investigate whether non-recurrent chromosomal rearrangements in proximal 17p represent random events or reflect susceptibilities to DNA rearrangements due to genome architecture, we analyzed the breakpoints of 18 unusual-sized deletions and eight balanced and unbalanced chromosome translocations involving proximal 17p.

Material and Methods

Chromosome Rearrangements

On the basis of the absence of the ~1.1-Mb junction fragment in PFGE analysis (Chen et al. 1997), we selected 16 patients with SMS who have unusual-sized deletions (patients 147, 540, 566, 572, 641, 993, 1153, 1190, 1195, 1354, 1456, 1615, 1774, 1931, 1939, and 2011) and two patients without the major features of SMS with deletions involving the SMS-common deletion chromosome region (patients 357 and 765) for this study. In addition, eight cell lines from patients with balanced or unbalanced chromosome translocations involving 17p11 were studied (table 1). Peripheral blood samples from patients and family members were obtained after informed consent.

Chromosome Breakpoint Mapping

The BAC and PAC clones used for the chromosome breakpoint mapping were identified on the physical maps of the regions of interest (Inoue et al. 2001; Bi et al. 2002; National Center for Biotechnology Information Home Page; UCSC Genome Bioinformatics Web site). Interspersed repeat sequences within the downloaded DNA sequence of the clones were eliminated by RepeatMasker (RepeatMasker Web Server) and were analyzed using Sequencher (Gene Codes) and NCBI BLAST (NCBI BLAST Home Page). The BAC/PAC clones were purchased from the BACPAC Resources Center and Research Genetics, and DNA was prepared from each through use of the PSI Clone BAC DNA kit (Princeton Separations) according to the manufacturer's instructions.

FISH

FISH was performed on metaphase and interphase cells of peripheral blood lymphocytes, Epstein-Barr virus-transformed peripheral blood lymphoblasts, and skin fibroblasts, as described by Shaffer et al. (1997). For chromosome 17 centromere identification, a directly labeled SpectrumGreen centromeric probe, D17Z1 (Vysis), was used.

FISH Screening for the SMS Common Deletions

A FISH assay with probes flanking the SMS-REPs was developed. For probes, we used proximal and distal

SMS-REP-flanking BAC/PAC clones: RP11-344E13 or RP11-98L14 (adjacent to the proximal SMS-REP on the centromeric side), RP5-836L9 (adjacent to the proximal SMS-REP on the telomeric side), RP11-416I2 (centromeric to the distal SMS-REP), and RP11-209J20 (telomeric and adjacent to the distal SMS-REP) (fig. 2). Clones flanking proximal SMS-REP were used in FISH analysis, and those flanking distal SMS-REP were used concurrently in a separate chamber on the same slide (fig. 2). When the smaller-sized deletion was identified, the middle SMS-REP-flanking BAC/PAC clones RP11-28B23 (centromeric to the middle SMS-REP) and RP11-178F10 (telomeric to the middle SMS-REP) were cohybridized, using FISH in interphase cells, to determine whether the middle SMS-REP was involved.

Genotyping

We determined both (1) the parental origin of the rearranged chromosomes and (2) distinguished inter- and intrachromosomal recombination mechanisms resulting in the deletion, using a combination of microsatellite haplotype reconstruction and the segregation of marker genotypes, on genomic DNA purified from peripheral blood (Gentra), as described by Shaw et al. (2002). Phases of parental haplotypes were defined on the basis of the most parsimonious explanation for observed genotypes in the siblings and under the assumption of no recombination.

Long-Range PCR

For the long-range PCR of the *KER* cluster, the following ≥ 30 -bp primers of 50% GC content and melting temperature 65–70°C were designed: F (CCGTGACTACAGCCAGTACTACAGGATAATCG) and R (CTCTGCAGTCTCCAGGACATAGATTTGCTC). The reaction was performed using the TaKaRa LA PCR Kit (Takara Shuzo), following the manufacturer's recommendations. Initial denaturation at 94°C for 15 min was followed by 40 cycles of denaturation at 98°C for 20 s, extension at 68°C for 10 min, and a final extension at 72°C for 10 min. The 4,134-bp product was extracted from the 1% agarose gel through use of a Gel Extraction Kit (Qiagen).

Somatic Cell Hybrids

We performed polyethylene glycol fusion between the lymphoblastoid cell line from patients 765 (Elsea et al. 1997) and 1153 and from a thymidine kinase-deficient (TK⁻) hamster cell line, A23 (Chen et al. 1997). For this fusion, 24 independent clones were isolated with cloning rings and were transferred to a 24-well microtiter plate. We obtained cells representing each clone by trypsinization of a confluent well of a 24-well plate, and we then transferred them to a 6-well plate and then to T25

Table 1

Summary of Findings in Patients with Chromosome Translocations Involving Proximal 17p

PATIENT	KARYOTYPE	BREAKPOINTS			CLINICAL INFORMATION
		Chromosome 17	Partner Chromosome		
GM03119 ^a	46,XX,t(9;17)(p22;q11.1)	Within D17Z1 in 17q11.1 (fig.4E)	Not mapped		A clinically normal woman with history of several spontaneous abortions
GM02836 ^a	46,XY,t(9;17)(q12;p11)[61%]/46,XY,t(9;17)(q12;p11),ins(3;1)(q21;q25q44)[39%]	Between RP11-728E14 and D17Z1 (fig. 4D)	Not mapped		A clinically normal individual
TTC90	46,XX,t(9;17)(q34.1;p11.2)mat, del(9)(q22.32q33.2)	Within two overlapping BAC clones: CTD-2354j3 and RP11-311F12	Between RP11-489N22 and RP11-88G17		An 11-year-old girl with Gorlin syndrome and features of nail-patella syndrome
UK	46,XY,t(1;17)(p36.3;p11.2)	Distal-most 1/3 portion of clone RP11-344E13	Within two overlapping PAC clones RP1-453P22 and RP1-505B13		A family in which nonsyndromic mental retardation and an apparently balanced reciprocal translocation segregated in eight individuals over three generations (Hussain et al. 2000)
1071	46,XX,t(X;17)(p22.3;p11.2)	Between RP11-728E14 and D17Z1	Distal to the most subtelomeric BAC clone RP11-215A12		A clinically normal 2-year-old girl, in whom balanced chromosome abnormality was found prenatally during amniocentesis (advanced maternal age)
1183	46,XY,t(2;17)(p25.3;p11.1)	Within distal-most ~1/4 portion of D17Z1 in 17p11.1	Within two overlapping BAC clones RP11-455M16 and RP11-163G21		A 9-year-old boy with mental retardation, in whom the balanced chromosome abnormality was found prenatally (advanced maternal age); a diagnosis of SMS has been excluded
1307	46,XY/46,XY,der(X)t(X;17)(p22.1;p11.1), ~50% mosaic	Within the middle of D17Z1	Not mapped		A patient with clinical and electrophysiological features of the CMT1A, in whom an extra PMP22 gene resulted from a rare unbalanced translocation of 17p to the X chromosome (King et al. 1998)
1576	46,XY,der(17)t(10;17)(q26.3;p11.2)	Within PAC clone RP1-48J12, at the centromeric end of the distal SMS-REP (fig 4E, 7)	Within overlapping BAC clones RP13-137A17 and RP13-439H18		A 5.5-year-old boy with the features of partial trisomy 17p and monosomy 10q26.3qter, including CMT1A

^a The skin fibroblast cell lines in patients GM03119 and GM02836 were purchased from Coriell Cell Repositories.

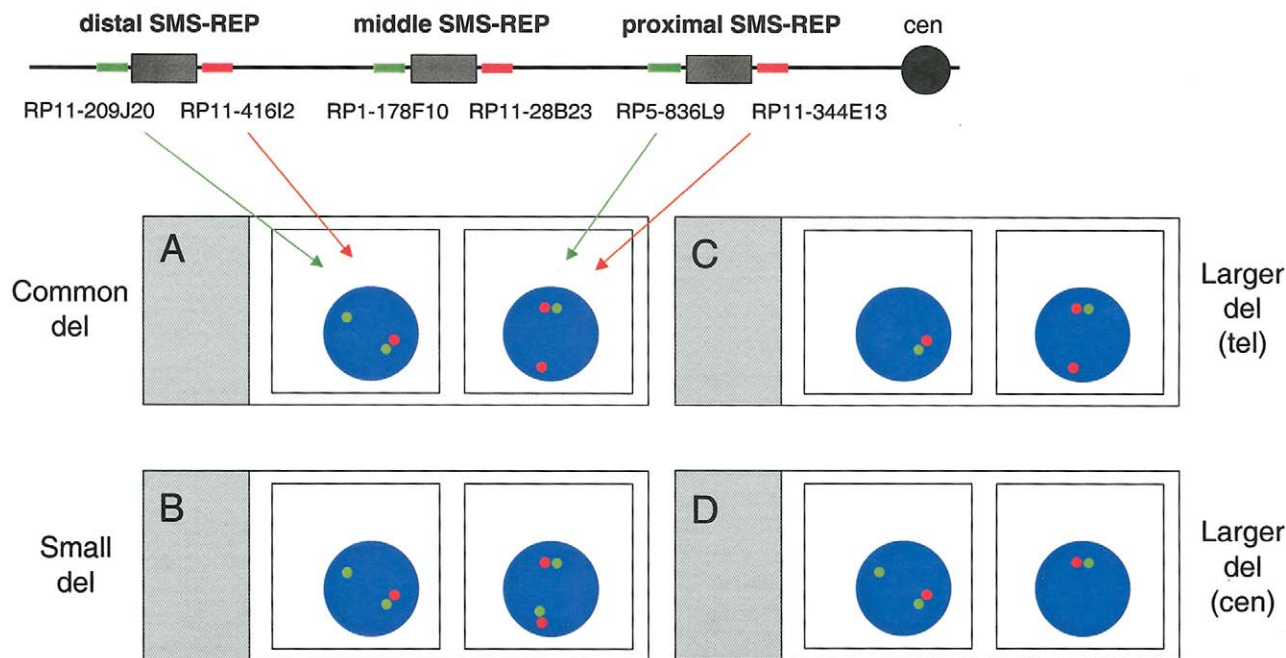


Figure 2 Schematic representation of a dual-color interphase FISH assay developed to screen for common, A, versus unusual-sized SMS deletions. The map of chromosome 17p11.2 with the placement of the FISH probes for one chromosome homologue is shown at the top of the figure. The proximal SMS-REP–flanking clones BAC RP11-344E13 and PAC RP5-836L9 and the distal SMS-REP flanking BACs RP11-416I2 and RP11-209J20 are differentially labeled and are detected with red and green colors, respectively. Below the chromosome map, in the left chamber of the slide, two adjacent green and red dots represent the normal chromosome 17, and the presence of a single green signal demonstrates that the deletion breakpoint occurred between clones RP11-209J20 and RP11-416I2, within the distal SMS-REP. Similarly, the absence of the second green signal on the right side indicates that the breakpoint maps between clones RP5-836L9 and RP11-344E13. The red and green signals flanking SMS-REPs do not overlap, because the distance between the clones is greater than the ~100-kb resolution limit of interphase FISH. The three other hypothetical microscope slides give examples of the FISH results obtained with the same clones. B, A small deletion with the telomeric breakpoint mapping within the distal SMS-REP and the centromeric breakpoint mapping between the proximal and distal SMS-REPs. C, A large deletion with the distal breakpoint mapping telomeric to the distal SMS-REP and the centromeric breakpoint mapping within the proximal SMS-REP. D, A large deletion with the telomeric breakpoint mapping within the distal SMS-REP and the proximal breakpoint mapping centromeric to the proximal SMS-REP.

flasks. Two-color FISH with probes mapping within and outside the deleted region was used to analyze the hybrids and identify those with the 17p11.2 deletion chromosome.

PFGE Analysis

High-molecular-weight DNA was isolated in agarose plugs from peripheral blood samples, somatic cell hybrid cell lines, and lymphoblastoid cell lines established from patients and controls (Pentao et al. 1992). For Southern analysis, we used the 1.1-kb *HindIII* fragment from the cDNA clone 41G7A, which contains the 3' end of the coding region and part of the 3' UTR, as the *CLP* probe; and we used the PCR product (F: ATGTCGGTTTGGG-TGTTTGT; R: TTAAGCACTTGGCTCAAGCA) of the *PRPSAP2* gene, localized adjacent to the middle SMS-REP on the centromeric side within the BAC clone RP11-28B23, as the *PRPSAP2* probe.

DNA Sequence Analysis

The search for additional LCRs was performed using NCBI BLAST analysis against the high-throughput and the nonredundant sequence database, and the sequence was assembled using NCBI BLAST 2 and the Sequencher software (Gene Codes).

Results

A Novel FISH Assay for Distinguishing the Common versus Unusual SMS Deletions

To replace technically challenging and time-consuming PFGE-based screening for the SMS common versus nonrecurrent deletions, we developed a novel FISH assay (fig. 2). Using dual-color interphase FISH with proximal SMS-REP–flanking BAC/PAC clones RP11-344E13 and RP5-836L9 and concurrently using the distal SMS-REP flanking BAC clones RP11-416I2 and RP11-209J20 (fig.

2), we were able to determine simultaneously whether the SMS deletion was of an unusual size and whether it was smaller or larger than the common deletion. The SMS-REPs are ~200 kb in size and thus larger than the ~100-kb resolution limit of interphase FISH. Therefore, cohybridized SMS-REP-flanking clones can be visualized as distinct signals by FISH. Direct FISH on uncultured cells can be used, because this approach does not require metaphase chromosomes, thus enabling rapid analyses. Compared with the PFGE analysis (Chen et al. 1997), this novel FISH approach is an easier, faster, much less expensive, and at least equally reliable method for screening for common SMS deletions.

Of note, two of the SMS-REP-flanking BAC clones, RP11-416I2 and RP11-344E13, contain the 33-kb and 23-kb fragments homologous to SMS-REP; however, the size of these segmental duplications did not affect the interpretation of the FISH studies. If a smaller-sized deletion is identified, the middle SMS-REP-flanking BAC clone RP11-28B23 (centromeric to the middle SMS-REP), together with the PAC RP1-178F10 (telomeric to the middle SMS-REP) (fig. 2), can be cohybridized to determine whether the middle SMS-REP is involved in the deletion. Moreover, the same FISH approach can be used for screening of the common duplication, dup(17)(p11.2p11.2) (Potocki et al. 2000). In addition to identifying the common duplication, it will reveal whether the fragment is direct or inverted in orientation.

Uncommon Deletion Breakpoints Map within LCRs

To investigate the recombination products for the unusual-sized deletions, we mapped both breakpoints for each. Surprisingly, we found that 13 of the 14 deletion breakpoints (in patients 147, 572, 765, 1153, 1190, 1456, and 1939) map within the recently identified LCRs: LCR17pA, LCR17pB, and LCR17pD (figs. 1 and 3). Moreover, FISH and PFGE results showed that eight deletions have one breakpoint mapping within proximal (patients 566, 993, 1615, 1774, and 2011), middle (patients 540 and 641), or distal (patient 1195) SMS-REPs (Bi et al. 2002) (figs. 1 and 3). The remaining three unusual deletions (patients 357, 1354, and 1931) do not have a breakpoint in any recognizable LCRs (figs. 1 and 3). These findings indicate that most (21/33) breakpoints of unusual-sized deletions in 17p11.2 may in fact be mediated by genome architectural features such as LCRs.

Three patients with larger-sized deletions (patients 147, 1153, and 1939) have distal breakpoints within LCR17pA and proximal breakpoints within LCR17pD (figs. 1 and 3). In these patients, we mapped the proximal breakpoint centromeric to the proximal SMS-REP, using FISH (figs. 1, 3, and 4A), and we mapped the distal breakpoint telomeric to the distal SMS-REP (figs. 1, 3, and 4B).

PCR on the somatic cell hybrid from patient 1153 indicated that the distal breakpoint occurred within BAC CTD-3157E16, and the proximal breakpoint within BAC RP11-218E15, at the centromeric ends of LCR17pA and LCR17pD, respectively (data not shown). BLAST analysis of the LCRs revealed that LCR17pA, LCR17pC, and LCR17pD are oriented in the same direction, whereas LCR17pB is inverted with respect to the other LCR17p copies. These data suggest that LCR17pA, together with directly repeated LCR17pD, may serve as substrates for NAHR, thus potentially explaining the apparent clustering, or recurrence, of deletion breakpoints in patients 147, 1153, and 1939.

The distal deletion breakpoint in patient 572 mapped within LCR17pA (fig. 4C), and the proximal deletion breakpoint mapped within the inverted LCR17pB copy. Two proximal deletion breakpoints (in patients 540 and 641) involved the middle SMS-REP (which is inverted with respect to other SMS-REPs) (figs. 1 and 3). In patients 1190 and 1456, the proximal breakpoints mapped within the LCR17pB copy, and the distal breakpoints involved the distal SMS-REP (Park et al. 2002). In support of this contention, PFGE experiments and Southern analysis in patients 540, 641, and 1456—through use of *PRPSAP2*, which maps centromeric and adjacent to the middle SMS-REP and LCR17pB, as a probe—identified junction fragments of 1.7-Mb, 0.9-Mb, and 1.1-Mb, respectively, in addition to the normal 1.4-Mb DNA fragment (fig. 5).

Six deletions have one breakpoint involving the proximal or distal SMS-REPs (in patients 566, 993, 1195, 1615, 1774, and 2011), and the other breakpoint appears to map within unique sequence. However, FISH analysis demonstrates the clustering of the distal breakpoints within one BAC clone in patients 566, 1354, 1774, and 1931 (RP11-45M22) and the clustering of proximal breakpoints in patients 1195 and 1931 (CTD-2010G8), suggesting a potential genome architectural feature stimulating these rearrangements, although analyses of the genomic sequence available at the time of writing (November 2002) failed to identify an obvious LCR or other higher-order sequence structure (figs. 1 and 3).

PCR analysis of a somatic cell hybrid retaining the deleted chromosome from patient 765 showed that the proximal breakpoint maps to a 100-bp interval (nucleotide position 87925–88024 bp) in the finished sequence BAC clone RP11-258F1. The precise mapping of the distal breakpoint within BAC CIT-3157E16 (LCR17pA) was hampered by the presence of the highly homologous and nondeleted LCR17pB sequences in this somatic cell hybrid (figs. 1 and 3). Since this patient did not manifest the typical features of SMS, the mapping of this breakpoint potentially narrowed the SMS critical region to 210 kb (Bi et al. 2002).

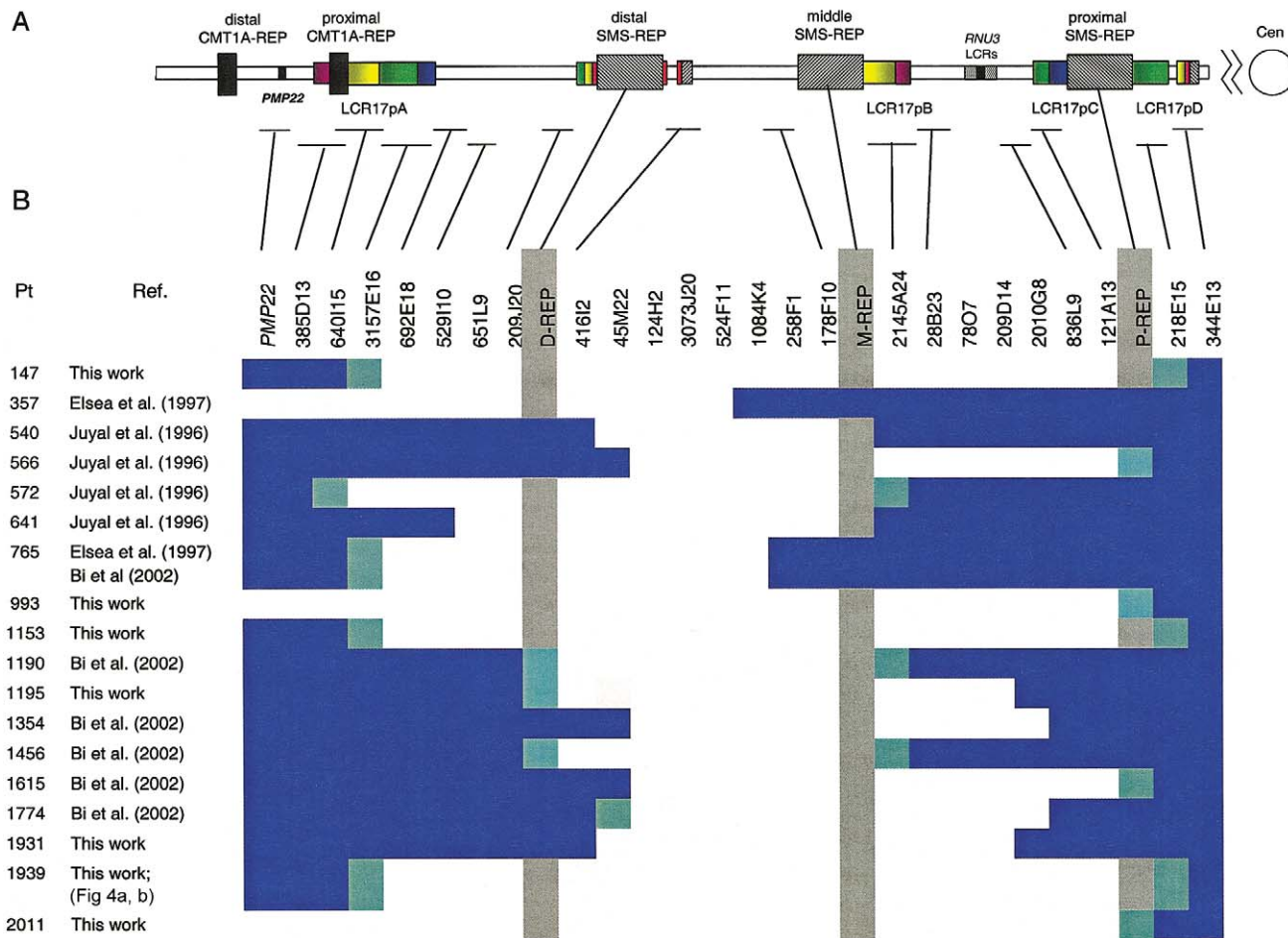


Figure 3 Summary of deletion breakpoint mapping. *A*, Schematic representation of LCRs within 17p11.2-p12, with horizontal lines attached to the table showing the position of individual BAC/PAC clones. *B*, A table with the clones used in FISH studies. PAC/BAC clones that gave positive FISH signals are represented by filled blue bars, and white spaces depict BAC/PAC clones that did not give a hybridization signal from the deleted chromosome (however, each individual clone was not assayed by FISH). Clones, within which the breakpoint was mapped, are depicted by half blue-green shading. The gray vertical shading indicates the three SMS-REPs. Selected clones used for the FISH analysis of uncommon deletions are taken from the complete BAC/PAC contig (Bi et al. 2002) and are labeled in the upper row of the table. Our analysis of the currently available databases reveals that clones RP11-416I2 and RP11-45M22 appear not to overlap and are spanned by the BAC clone RP11-367G9.

Unequal Crossing-Over as a Mechanism for Uncommon Deletions

Recently, the common ~4-Mb SMS deletion has been shown to occur via unequal meiotic crossing-over between the proximal and distal SMS-REP copies (Shaw et al. 2002). However, it is unknown whether deletions with uncommon breakpoints have arisen through the same mechanism. To investigate the genetic mechanism for generating uncommon deletions, we used microsatellite markers in patients and family members to reconstruct the haplotypes of five patients with SMS with unusual-sized deletions (patients 641, 1190, 1354, 1456, and 1931). Surprisingly, three deletions (in patients 641, 1190, and 1354) result from unequal interchromosomal

recombination occurring between the genetic markers flanking each breakpoint (fig. 6).

The deletions in patients 1190 and 1456 have the same breakpoints within the LCRs, distal SMS-REP, and LCR17pB. It is possible that, similar to the deletion in patient 1190, the deletion in patient 1456 may also result from an unequal crossing-over event but between chromatids and not chromosomes (i.e., it is intrachromosomal). However, because SMS-REP and LCR17pB blocks are not homologous to each other, the unequal crossing-over could result from either minor homology regions (e.g., repetitive sequences within the LCRs) or nonhomologous end-joining (NHEJ). Interestingly, such LCR-stimulated NHEJ events have been described in the

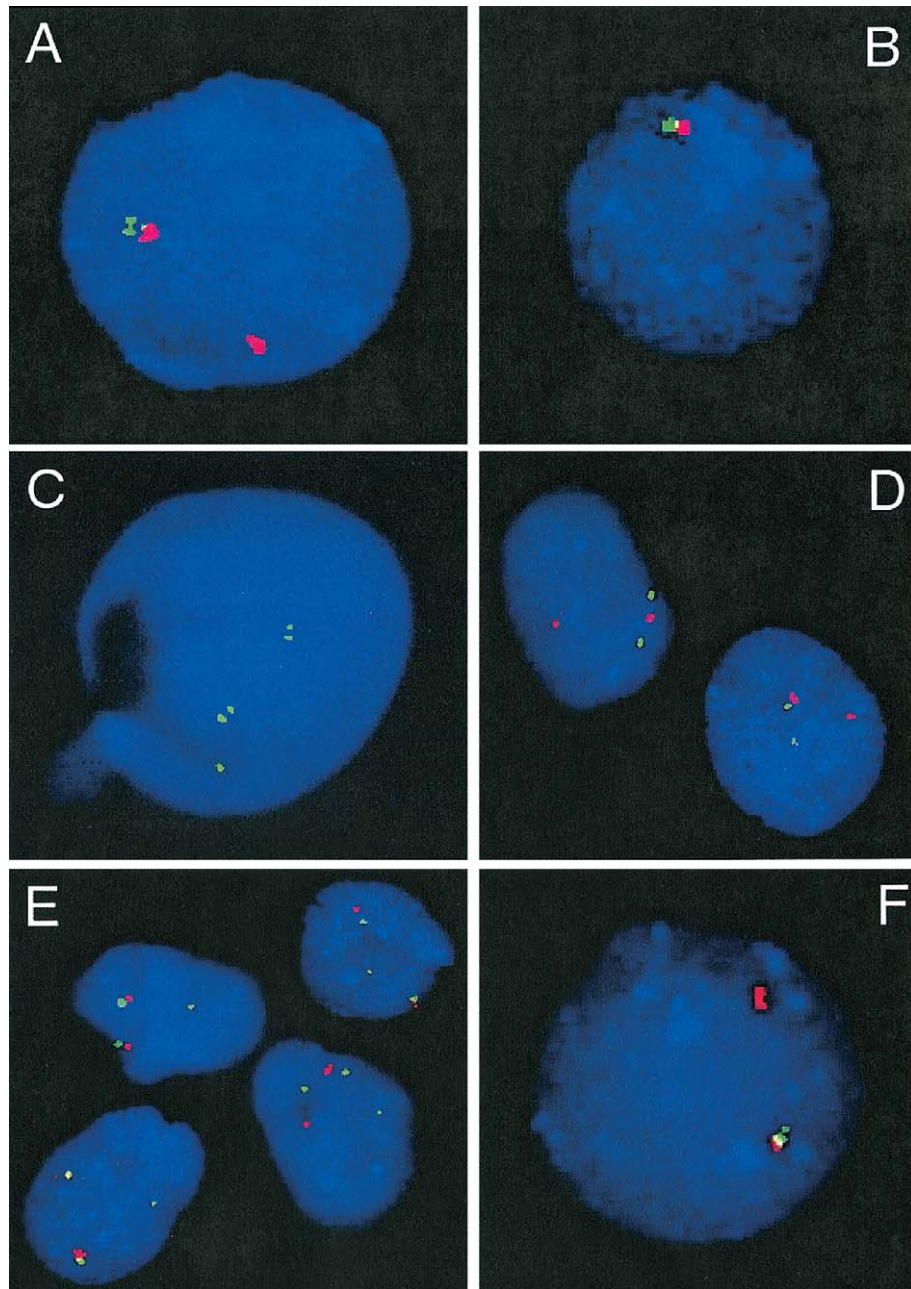


Figure 4 FISH analyses of interphase nuclei used to map the rearrangement breakpoints. *A* and *B*, Interphase nuclei of patient 1939 after FISH with SMS-REPs-flanking clones (fig. 2). In *A*, the absence of the green signal (RP5-836L9) and presence of the red signal (RP11-344E13) on the del(17) indicates that the proximal breakpoint maps within the proximal SMS-REP (or directly adjacent LCR17pB). In *B*, the absence of both red (RP11-416I2) and green (RP11-209J20) signals on the del(17) shows that the distal breakpoint maps telomeric to the distal SMS-REP. *C*, FISH with BAC clone CIT-3157E16 (LCR17pA), enabling mapping the distal breakpoint of the deletion in patient 572 to the distal portion of LCR17pA. The two closely spaced green signals on the normal and deleted chromosomes 17 represent LCR17pC and LCR17pD copies. A single hybridization signal corresponds to the LCR17pA copy on the normal chromosome 17; the LCR17pA on der(17) is deleted. *D*, FISH with the BAC RP11-344E13 (*red*) and a centromeric probe (*green*) on cells from patient GM02836, showing the breakpoint mapped between them. On the normal chromosome 17, the red and green signals are relatively close to each other, whereas the separation of the red and green signals indicates that they are on different chromosomes, der(9) and der(17), respectively. *E*, The centromeric breakpoint on chromosome 17 in the patient GM03119, identified after the cohybridization of BAC RP11-344E13 (*red*) and the centromeric probe D17Z1 (*green*). In addition to the adjacent red and green pair of signals on both chromosomes 17 and the der(9), the single green signal on the der(17) is of weaker intensity when compared with the other two green signals, indicating the localization of this breakpoint to the q11.1 portion of the chromosome 17 centromere. Note the variability of distances between RP11-344E13 (*red*) and the D17Z1 centromeric probe (*green*) in *D* and *E*, demonstrating different condensation of the pericentromeric heterochromatin. *F*, FISH with the distal SMS-REP flanking BAC clones RP11-209J20 (*green*) and RP11-416I2 (*red*) on an interphase nucleus of patient 1576, a carrier of an unbalanced translocation. The presence of only the red signal on the der(17) chromosome indicates that the breakpoint maps between these two clones.

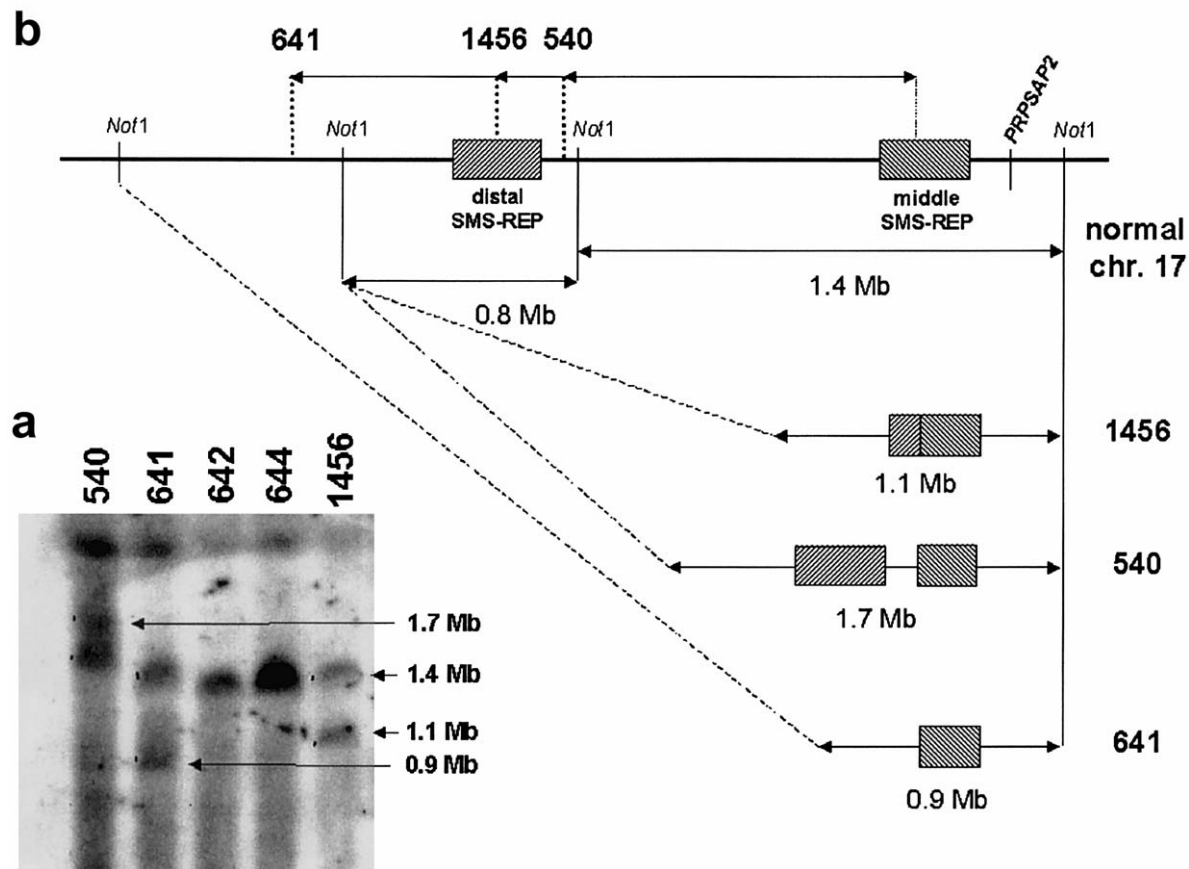


Figure 5 A, PFGE detection of novel junction fragments in three patients with SMS (patients 540, 641, and 1456) with smaller-sized deletions in which the proximal breakpoint maps to middle SMS-REP. The Southern blot was hybridized with a *PRPSAP2* PCR probe, which, in addition to the normal 1.4-Mb *NotI* fragment (control patients 642 and 644), identified the 0.9-, 1.1-, and 1.7-Mb junction fragments (arrows) spanning the proximal breakpoints within middle SMS-REP or LCR17pB. B, Schematic diagram represents the derivation of novel PFGE junction fragments in patients with SMS with uncommon deletions.

very rare group of patients with Pelizaeus-Merzbacher disease, resulting from chromosome deletions (and not duplications) involving genomic segments containing the causative gene *PLP1* (Inoue et al. 2002). The same end-joining mechanism following unequal crossing-over events between LCR-free unique-sequence DNA fragments could be responsible for the origin of smaller-sized deletions in patient 1354 (interchromosomal) and possibly 1931 (intrachromosomal).

Interestingly, of 14 analyzed cases, 12 deletions (in patients 572, 641, 1153, 1190, 1195, 1354, 1456, 1615, 1774, 1931, 1939, and 2011) were of paternal origin and two (in patients 540 and 993) were maternal in origin ($P \leq .013$).

Nonrecurrent Translocation Breakpoints Cluster at the Chromosome 17 Centromere

To determine whether nonrecurrent chromosome translocation breakpoints are associated with genome

architecture and potential susceptibility to breakage, we investigated the breakpoints of eight nonrecurrent, reciprocal chromosome translocations (table 1). Unexpectedly, we found six breakpoints clustered between the proximal SMS-REP and the chromosome 17 centromeric α -satellite sequence D17Z1; one between proximal and middle SMS-REPs within two overlapping BAC clones, CTD-2354J3 and RP11-311F12; and one at the centromeric end of the distal SMS-REP (fig. 7; table 1). Using BLAST, the Golden Path physical map, and interphase FISH analyses, we estimated the size of this pericentromeric segment as <1 Mb and the distance between the most proximal BAC clone RP11-728E14 and the centromere as <0.5 Mb (fig. 7). Of note, using dual color interphase FISH with the BAC clone RP11-344E and the α -satellite centromeric probe D17Z1, we observed a large variability in the distance between the signals, indicating significant differences in the DNA condensation of this region or polymorphic variation in

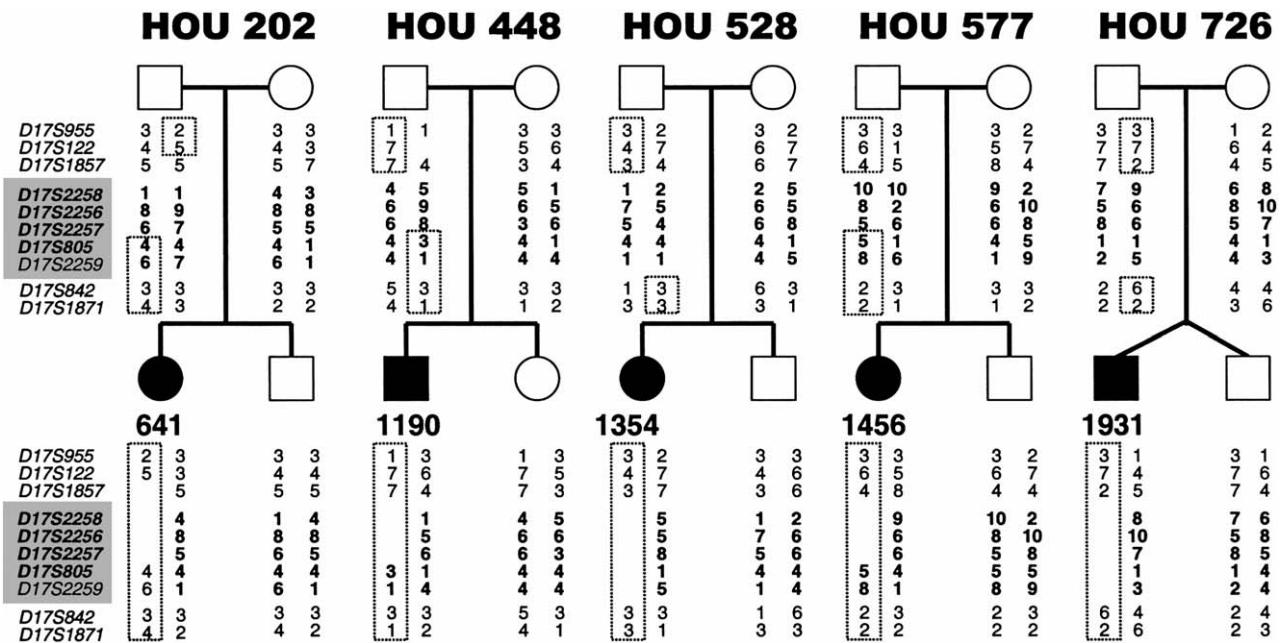


Figure 6 Haplotypes of five patients with unusual deletions and their families. Standard pedigree symbols are used; a circle denotes a female, a square denotes a male. Blackened circles or squares indicate an affected individual. To the left of each pedigree is a list of microsatellite markers used for genotyping; those within the SMS common deletion region are bold and shaded. The allele numbers are located under each family member. The genotypes of markers within the SMS common deletion region are bold in the patients and the parent of origin. The dotted lines outline alleles inherited by the patient from the parent of origin. In patient 641, recombination occurred between the region flanked by loci D17S122 and D17S1857 and the region between D17S2257 and D17S805 (including the middle SMS-REP), resulting in the deletion. In patient 1190, recombination between the region flanked by markers D17S1857 and D17S2258 (including the distal SMS-REP) and the region flanked by markers D17S2257 and D17S805 (including the middle SMS-REP) resulted in the deletion. Recombination between the region flanked by loci D17S1857 and D17S2258 and the region between D17S2259 and D17S842 (including the proximal SMS-REP) resulted in the deletion in patient 1354. Patients 1456 and 1931 may have deletions resulting from intrachromosomal recombination. (Both of these patients had crossovers on their intact, maternally derived chromosomes 17 [between loci D17S842 and D17S1871 for patient 1456 and between loci D17S955 and D17S122 for patient 1931]). Interestingly, each of the five deletions are paternally derived, as evidenced by the lack of a paternal allele for loci D17S1857, D17S2258, D17S2256, and D17S2257 for patient 641; loci D17S2258, D17S2256, and D17S2257 for patients 1190 and 1456; and loci D17S2258, D17S2256, D17S2257, D17S805, and D17S2259 for patients 1354 and 1931. The locations of markers used in genotyping are shown in figure 1.

pericentromeric/centromeric sequence size (fig. 4D and 4E). Interestingly, four of eight partner chromosome breakpoints mapped within the most telomeric subbands: 1p36.3, 2p25.3, 10q26.3, and Xp22.3.

LCRs Comprise at Least 23% of Proximal 17p Genomic Sequence

Estimates of the percentage of low-copy repeat sequence in the human genome have varied from 5% to 10% (Mazzarella and Schlessinger 1998; Bailey et al. 2001; Cheung et al. 2001; Eichler 2001; IHGSC 2001; Johnson et al. 2001; Samonte and Eichler 2002) and are based on the virtual analyses of an ever-changing (version 30, August 2002) draft of the human genome. The size and percent identity of LCR necessary and sufficient to mediate genomic disorders remain to be elucidated, but the usual minimal size for an LCR associated with a large genomic segment rearrangement is ~10 kb with ~98% sequence identity (Lakich et al. 1993; Lupski

1998; Bailey et al. 2001; Stankiewicz and Lupski 2002a). Extensive studies of proximal 17p rearrangement breakpoints in combination with nearly complete genome sequence enable estimates of the percent genomic sequence contained within LCR, based on experimental observations. Remarkably, in the 7.4 Mb of proximal 17p genomic sequence from the centromere to the distal CMT1A-REP, at least 1.7 Mb (23%) of the genome is contained within LCRs. These LCRs include the proximal and distal CMT1A-REPs (24,011 bp) (Reiter et al. 1997); proximal (~256 kb), middle (~241 kb), and distal (~176 kb) SMS-REPs; LCR17pA (~383 kb), LCR17pB (~191 kb), LCR17pC (~91 kb), LCR17pD (~118 kb) (Park et al. 2002), LCR17pE (~31 kb), LCR17pF (~33 kb), and LCR17pG (~23 kb); at least two inverted repeats flanking the *RNU3* gene (2 × ~45 kb) (Gao et al. 1997); and three LCRA in and around the CMT1A region (3 × ~11 kb) (Inoue et al. 2001) (fig. 1), the majority of which (with the exception of LCRA, LCR17pC, and

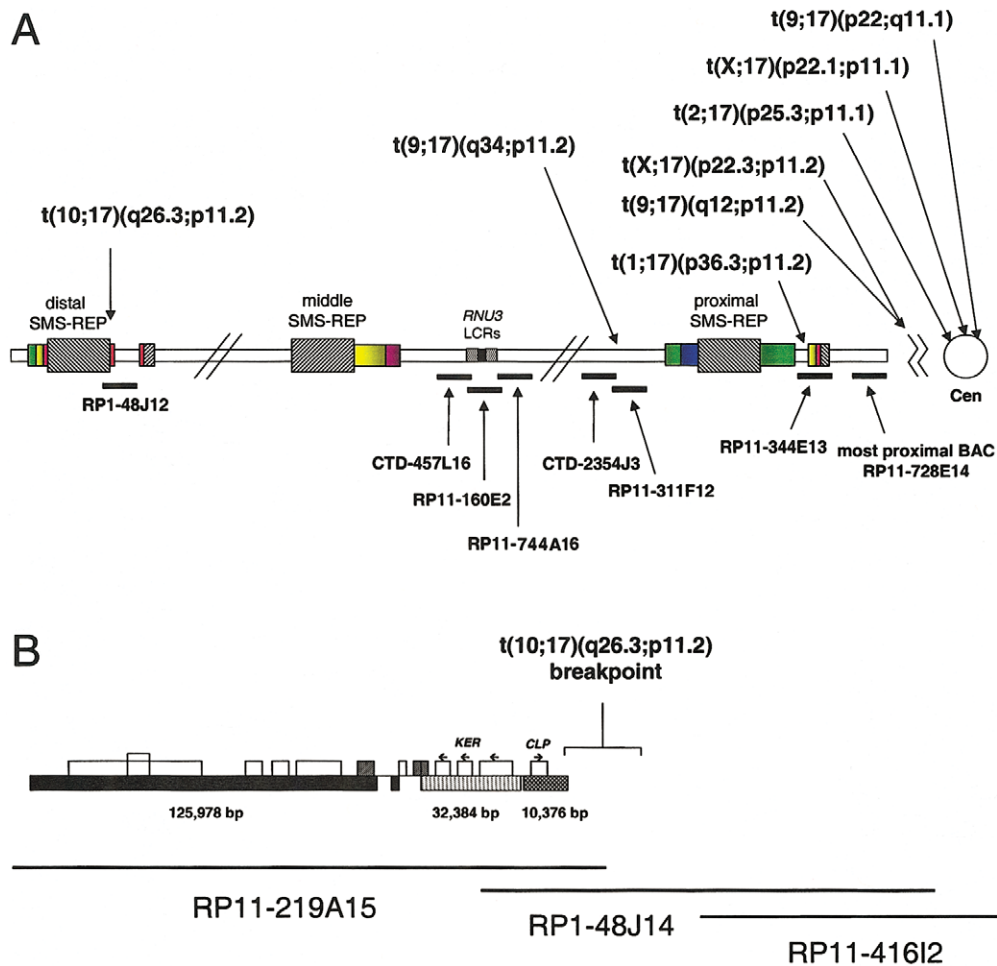


Figure 7 A, Schematic diagram of identified translocation breakpoints within proximal 17p. FISH experiments on cells from patients harboring translocations with breakpoints in proximal 17p (table 1) showed that five of eight analyzed breakpoints cluster centromeric to the most proximal BAC clone RP11-728E14: one within clone RP11-344E13, one within two overlapping BAC clones (CTD-2354J3 and RP11-311F12), and one at the centromeric end of the distal SMS-REP, within the PAC clone RP1-48J12. B, Schematic representation of the chromosome 17 translocation breakpoint in patient 1576. The breakpoint was mapped between the BAC clones RP11-416I2 and RP11-209J20, indicating that it occurred within or adjacent to the distal SMS-REP on the centromeric side. FISH with the long-range PCR product specific to the *KER* gene cluster localized within the ~10–42-kb proximal portion of the distal SMS-REP (Park et al. 2002) showed that it was translocated on the der(10) chromosome. Subsequent FISH mapping with the PAC clone RP1-48J12 that overlaps the distal SMS-REP by ~20 kb showed that only a small fragment of the clone RP1-48J2 was translocated. Thus, the chromosome 17 breakpoint was mapped at the proximal end of the distal SMS-REP.

RNU3 repeats) have been identified at the breakpoints of the rearranged chromosomes.

Discussion

Genome Architecture and Susceptibility to Nonrecurrent Rearrangements in Proximal Chromosome 17p

In contrast to recurrent common chromosome aberrations, in which the breakpoints are associated with various genomic architectural features such as LCRs, AT-rich palindromes, or fragile sites, the unusual-sized nonrecurrent rearrangements were thought to represent ran-

dom events. To investigate this hypothesis, we studied 26 nonrecurrent chromosome rearrangements involving proximal 17p. Surprisingly, we found that many of the unusual-sized deletions and chromosome translocations have breakpoints clustering within apparently breakage/recombination-prone genome architectural structures.

Recently, the construction and DNA sequence analysis of the complete BAC/PAC contig covering the CMT1A and SMS common deletion regions within chromosome 17p11.2-p12, in combination with patient breakpoint analysis, have enabled us to identify the novel LCR17p structures (Inoue et al. 2001; Bi et al. 2002; Park et al. 2002). We now show that LCR17pA, LCR17pC, and

LCR17pD are in a direct orientation with respect to each other, whereas the fourth copy, LCR17pB (adjacent to the middle SMS-REP on the centromeric side), is inverted. Remarkably, each of these repeats (except LCR17pC), as well as middle SMS-REP, has now been mapped to the breakpoints of rearranged chromosomes.

We have demonstrated that, similar to the recurrent common genomic deletions and duplications in several other contiguous gene syndromes (Emanuel and Shaikh 2001; McDermid and Morrow 2002; Stankiewicz and Lupski 2002a), LCRs may also underlie nonrecurrent rearrangements. We found that the breakpoints of three larger-sized SMS deletions mapped within directly oriented LCRs. Like the SMS-REPs and CMT1A-REPs, these newly identified LCRs in proximal 17p also appear to serve as genomic substrates mediating NAHR, resulting in chromosome rearrangements.

On the basis of the presence of the same junction fragment as shown by PFGE analysis, Chen et al. (1997) reported several patients with SMS with common deletions, and Potocki et al. (2000) described seven unrelated patients with duplication 17p11.2, the reciprocal product of the common SMS deletions. Recently, Shaw et al. (2002) demonstrated that both common SMS deletions, as well as reciprocal duplications flanked by the proximal and distal SMS-REP, result from unequal crossing-over events with no parental origin bias. These genetic data further support the model of NAHR-mediated reciprocal deletion/duplication events and indicate the relevance of genome architectural features such as LCRs (SMS-REPs) in the origin of recurrent DNA rearrangements within proximal 17p. We now show that unusual-sized deletions can also result from unequal crossing-over events, suggesting they may be stimulated by the presence of some yet-unidentified LCRs. Supporting this hypothesis, the LCRs flanking the *RNU3* gene (localized between proximal and distal SMS-REPs) (figs. 1 and 3) were identified by molecular methods (Gao et al. 1997) but are yet to be identified in the nearly complete DNA sequence of the SMS region. Interestingly, similar to CMT1A (Palau et al. 1993) and spinal muscular atrophy (Wirth et al. 1997), 12 of 14 unusual-sized deletions analyzed by genotyping (in patients 572, 641, 1153, 1190, 1195, 1354, 1456, 1615, 1774, 1931, 1939, and 2011) were of paternal origin, suggesting a potential increased proclivity to deletions in this region during spermatogenesis.

In addition to the patients with the common duplication dup(17)(p11.2p11.2) (Potocki et al. 2000; Shaw et al. 2002), we have identified several individuals (Roa et al. 1996) with unusual-sized duplications (FISH-positive for duplication, but junction fragment-negative in PFGE screening using the *CLP* probe) involving this genomic region. We suggest that some of these duplications

may also result from LCRs/NAHR-based mechanisms, and this hypothesis is currently under investigation.

Nonrecurrent Translocation Breakpoints Cluster at the Chromosome 17 Centromere

Centromeres and the pericentromeric intervals of the human genome still remain among the greatest DNA sequencing challenges for the Human Genome Project. The border between centromeric heterochromatin and euchromatin is significantly enriched (10-fold) with various repetitive elements (Eichler et al. 1997; Eichler 1999; Bailey et al. 2001; Horwath et al. 2001; IHGSC 2001), thus making both the physical mapping and computational assemblies challenging (Katsanis et al. 2001).

Our complete BAC/PAC contig over the entire chromosome subband 17p11.2 (Bi et al. 2002) apparently ends <0.5–1 Mb from the centromere. Because of this proximity, we were able to identify the cluster of translocation breakpoints between proximal SMS-REP and the chromosome 17 centromere. We found that three of eight translocation breakpoints involving 17p11 were located within the α satellite, three others were located within an ~1-Mb segment from the centromere, and one was located within the distal SMS-REP. We suggest that the identified (peri)centromeric clustering of constitutional chromosome 17p11 translocation breakpoints may be associated with the observed variability of the DNA condensation (patients GM02836 and GM03119) of this genomic region (fig. 4D and 4E). Such decondensation of the pericentromeric heterochromatin has been proposed as a mechanism leading to the origin of jumping translocations of chromosome 1q in multiple myeloma (Sawyer et al. 1998). The abnormal condensation may in turn be related to the abundance of the LCRs in the hetero-euchromatin transition or histone modification (Horvath et al. 2001; Briggs and Strahl 2002).

Interestingly, in addition to isochromosomes (Mertens et al. 1994) and Robertsonian translocations (Han et al. 1994; Page et al. 1996; Bandyopadhyay et al. 2001), several chromosome breakpoints of different genomic rearrangements have been found to map within the centromere or within pericentromeric regions (Wolff et al. 1996; Tümer et al. 1998; Berger et al. 1999; Beheshti et al. 2000; Fauth et al. 2001). Until now, only a few reciprocal chromosome translocation breakpoints have been shown to be associated with LCRs. AT-rich palindromes within an LCR on 22q11 are responsible for the most common recurrent non-Robertsonian constitutional translocation in humans, resulting in the der(22)t(11;22) syndrome (Kurahashi et al. 2000; Edelman et al. 2001). Kehrer-Sawatzki et al. (1997) reported a reciprocal t(17;22)(q11;q11) in a family with neurofibromatosis type 1 with a breakpoint mapping to

the same AT-rich sequence in the same LCR, and Rhodes et al. (1997) described a translocation, t(1;22), involving LCR22. Recently, Giglio et al. (2002) demonstrated that t(4;8)(p16;p23), probably the second-most-common recurrent reciprocal translocation in humans after the t(11;22) translocation, is also mediated by the LCRs that consist of an olfactory receptor–gene cluster. Similar to our findings, the chromosome breakpoints of 14 different reciprocal translocations involving chromosome 22q11 were reported recently (Morrow et al. 2002). These authors found that five breakpoints mapped within LCR22-3, and an additional four occurred within the vicinity of other LCR22s. Interestingly, all 14 partner chromosome breakpoints mapped within the most telomeric bands.

Similar to constitutive (germline) rearrangements, somatic genomic events (e.g., isochromosome 17q, frequently found in patients with neoplasias such as leukemia and medulloblastoma) also have been proposed to involve genome architectural features such as LCRs (Fioretos et al. 1999; Scheurlen et al. 1999). In support of this notion, Saglio et al. (2002) recently reported a possible involvement of the 76-kb LCR22 in the origin of the Philadelphia chromosome translocation, t(9;22)(q34;q11.2). Mitotic rearrangement events present a challenge for breakpoint analyses because of tissue and cell mosaicism, and thus they likely remain underascertained.

We identified one translocation breakpoint (in patient 1576) mapping within or just adjacent and centromeric to the distal SMS-REP. Interestingly, this breakpoint is localized in the direct vicinity of the evolutionarily unstable portion of the distal SMS-REP. Recently, Park et al. (2002) proposed that the proximal SMS-REP was the progenitor copy that, through several genomic rearrangements 40–65 million years ago, resulted in the middle and the distal SMS-REPs. The evolutionary inversion of the entire proximal SMS-REP, generating the middle SMS-REP copy, was accompanied by the truncation of the terminal ~14-kb genomic interval including the *CLP* gene. An interstitial ~39-kb deletion of the genomic segment between the *KER* and *CLP* loci was one of the rearrangements associated with the origin of the distal SMS-REP (fig. 7B). These data, together with the identified localization of the t(10;17)(q26.3;p11.2) breakpoint within or directly adjacent to the *KER-CLP* portion of the distal SMS-REP, further suggests that this genomic interval containing the SMS-REPs is unstable and prone to rearrangements.

Genome Architecture and Rearrangements

LCRs have been recognized relatively recently, because of their association with DNA rearrangements resulting in disease traits and, in contrast to interspersed repeat

sequences (e.g., *Alu* or LINE), are not identifiable through reassociation kinetics. Nevertheless, the involvement of LCRs in chromosome rearrangements and evolution has received widespread attention (Lupski 1998; Bailey et al. 2001; Emanuel and Shaikh 2001; Johnson et al. 2001; Inoue and Lupski 2002; Samonte and Eichler 2002; Stankiewicz and Lupski 2002a, 2002b). Estimates of the amount of human genomic sequence contained within LCRs have ranged from 5% to 10% (Bailey et al. 2001; Johnson et al. 2001), but, to date, they have been based on the bioinformatic analyses of the draft genome sequences. Assembly of the human genome sequence (IHGSC 2001)—and, in particular, that determined by a shotgun approach—is challenging because of such LCRs (Lupski 1998; Katsanis et al. 2001), and the present genome content of LCRs may be grossly underestimated. Our analysis of the genome sequence in proximal 17p suggests that LCRs may constitute >23% of primary DNA sequence in some parts of the human genome. Furthermore, we demonstrate that each identified LCR within proximal 17p can be involved in a rearrangement event. In fact, 21/33 (64%) deletion breakpoints mapping within 17p11.2 occur in LCRs. This indicates the breakage/recombination–stimulating role of LCRs.

On the basis of our FISH and genotyping findings, we propose three different mechanisms resulting in deletion rearrangements: (i) Similar to common SMS deletions, the unusual-sized deletions with breakpoints mapping within directly oriented copies of LCRs (patients 147, 1153, and 1939; ~17%) are stimulated by LCRs and mediated by the LCR/NAHR–based inter- or intrachromosomal unequal crossing-over. (ii) In the other group of unusual-sized deletions, represented by those with both breakpoints mapping within nonhomologous copies of LCRs (patients 572, 1190, and 1456; ~17%) or those with one breakpoint mapping within LCR and the other in LCR-free unique DNA sequence (patients 540, 566, 641, 765, 993, 1195, 1615, 1774, and 2011; 50%), the chromosome deletion is stimulated—but not mediated—by the LCR(s) and may occur via either NAHR utilizing small repeat segments or by NHEJ (Inoue et al. 2002). (iii) Finally, deletions, in which breakpoints do not appear to involve LCRs (patients 357, 1354, and 1931; ~17%) may occur through NHEJ between repeat-free DNA fragments. It is possible that the completion of the DNA sequence of this region will reveal the presence of yet unknown additional low- or high-copy repeats at the apparently unique sequence breakpoints. Interestingly, the distal breakpoints of the deletions in patients 1354 and 1931 appear to cluster with the breakpoints in patients 566 and 1774, suggesting the presence of a breakage-prone genomic architectural feature.

Our findings in proximal 17p and the recent data from 22q11.2 reported by Morrow et al. (2002) document

that genome architecture is important to recurrent chromosomal rearrangements, including interstitial deletions and reciprocal translocations. Further studies are required to determine the extent to which LCRs influence susceptibility to chromosome rearrangements in other regions of the genome. Nevertheless, as in the case of recurrent rearrangements, the findings in proximal 17p (Pentao et al. 1992; Chen et al. 1997) may model what will be found in many other genomic regions.

Recombination-based Disorders and Disease Burden

Unlike conventional monogenic diseases reflecting errors of DNA replication and/or repair, genomic disorders are recombination-based conditions and thus cannot be prevented/repared by any cellular machinery (Lupski 2003). This has been proposed as a possible explanation for the high frequency and worldwide prevalence of new mutations in genomic disorders (Lupski 1998; Shaffer and Lupski 2000). Depending on the size of the genomic segment involved in the genomic disorder, it can result in a Mendelian disease, a contiguous gene syndrome, or a chromosomal disorder (Stankiewicz and Lupski 2002a). A wide variety of traits have been described as resulting from genomic disorders, including mental retardation, color blindness, hypertension, infertility, panic and phobic disorder, and peripheral neuropathy (Stankiewicz and Lupski 2002b).

In summary, our data demonstrate that genomic architecture involving LCRs plays a significant role in the origin not only of recurrent common chromosome rearrangements (e.g., contiguous gene syndromes) but also of unusual-sized deletions and nonrecurrent translocations.

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Electronic-Database Information

The URLs for data presented herein are as follows:

BACPAC Resources Center Home Page, Children's Hospital Oakland, <http://www.chori.org/bacpac/>
 Coriell Cell Repositories, <http://locus.umdj.edu/>
 NCBI BLAST Home Page, <http://www.ncbi.nlm.nih.gov/blast/>
 NCBI Home Page, <http://www.ncbi.nlm.nih.gov/>

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for SMS)
 RepeatMasker Web Server, <http://repeatmasker.genome.washington.edu/cgi-bin/RepeatMasker>
 Research Genetics, <http://www.resgen.com/index.php3>
 UCSC Genome Bioinformatics, <http://genome.ucsc.edu/>

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