Construction of a 780-kb PAC, BAC, and Cosmid Contig Encompassing the Minimal Critical Deletion Involved in B Cell Chronic Lymphocytic Leukemia at 13q14.3

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A putative tumor suppressor gene involved in B cell chronic lymphocytic leukemia (B-CLL) was mapped to human chromosome 13q14.3 close to the genetic markers D13S25 and D13S319. We constructed a 780-kb-long contig composed of cosmids, bacterial artificial chromosomes, and bacteriophage P1-derived artificial chromosomes that provides essential information and tools for the positional cloning of this gene. The contig contains both flanking markers as well as several additional genetic markers, three ESTs, and one potential CpG island. In addition, using one B-CLL patient, we characterized a small internal deleted region of 550 kb. Comparing this deletion with other recently published deletions narrows the minimally deleted area to less than 100 kb in our physical map. This deletion core region should contain all or part of the disrupted in B cell malignancies tumor suppressor gene.

INTRODUCTION

B cell chronic lymphocytic leukemia (B-CLL) is the most common hematological malignancy in western Europe, accounting for 30% of all leukemia cases. Cytogenetic analysis has revealed karyotypic abnormalities in approximately 50% of the reported B-CLL patients. Two cytogenetic abnormalities are quite frequent, trisomy 12 and structural abnormalities of chromosome 13, with each of them being found in approximately 25% of the B-CLL cases (Gahrton et al., 1980; J uliusson and Gahrton, 1990; Peterson et al., 1992).

Most of the chromosome 13 abnormalities are deletions of band q14.3, a locus containing the retinoblastoma tumor suppressor gene (RB). Although monoallelic losses of this gene have been described, the retained allele of RB remains functional in many B-CLL patients (Liu et al., 1993). The hypothesis of a second tumor suppressor gene located near RB has been put forward. Several studies have shown that a locus, near genetic marker D13S25 and telomeric to RB, resides in a critical region of chromosome 13 that is frequently deleted in B-CLL (Brown et al., 1993; Bullrich et al., 1996; Chapman et al., 1994; Devilder et al., 1995; Hawthorn et al., 1995; Liu et al., 1995) and contains the putative tumor suppressor gene termed DBM, for disrupted in B cell malignancies (Brown et al., 1993).

In preliminary mapping experiments, it was previously shown that the DBM locus resides at least 530 kb from the 3′end of the RB gene (Brown et al., 1993). More recent results, obtained by deletion mapping, localized the critical region of the deletion (Bullrich et al., 1996; Devilder et al., 1995; Liu et al., 1995; Stilgenbauer et al., 1995). Apparent discrepancies in these results assign the core region either between markers D13S294 and D13S272 (Devilder et al., 1995; Bullrich et al., 1996) or between markers D13S25 and D13S273 (Liu et al., 1995; Stilgenbauer et al., 1995), with a major deletion hot spot near marker D13S319 (Liu et al., 1995) or between markers D13S273 and D13S272 (Lichter et al., 1996).

To map the critical region definitively and to identify candidate tumor suppressor genes, we have constructed a contig covering the D13S25 region. Here we report the construction of a contig composed of cosmids, bacterial artificial chromosomes (BACs), and bacteriophage P1-derived artificial chromosomes (PACs) in which we have localized a number of genetic markers, three ESTs, and one CpG island. We also show, by fluorescence in situ hybridization (FISH) using members of the contig, and by testing loss of heterozygosity...
in the D13S25 region, that a short region flanked by markers AFMA301WB5 and D13S294 should contain all or part of the DBM gene, since it is the minimal deletion we have found in our B-CLL panel.

MATERIALS AND METHODS

DNA preparation. Cosmid, PAC, and BAC DNAs were prepared using Qiagen 500 columns (Qiagen GmbH, Hilden, Germany) according to the manufacturer’s instructions, except that volumes of buffers P1, P2, and P3 were increased. Yeast artificial chromosome (YAC) DNA for pulsed-field gel electrophoresis (PFGE) analysis was prepared from yeast cells and embedded in agarose block as previously described (Schwartz and Cantor, 1984).

Inter-Alu polymerase chain reaction of YAC clones. Inter-Alu polymerase chain reaction (PCR) was performed using the 5’ and 3’ human Alu PCR primers (TC65 or S17) and reaction conditions as previously described by Nelson et al. (1989). The reaction was carried out using 50 ng of total yeast DNA. The PCR products from the YAC were isolated using Magic PCR prep columns (Promega, Madison, WI). In two cases, prominent inter-Alu PCR fragments (I1A3/4 and I1Aa) were purified from agarose gels after electrophoresis.

Pulsed-field gel electrophoresis. All gels were made up in 0.5× TBE buffer with 1% fastlane agarose (Tebu, Plymouth Meeting, PA). Agarose plugs were digested in 200 μl of 1× restriction buffer at the temperature specified by the manufacturer for 4 h. Running conditions varied with fragment sizes being resolved, but consisted of continuously ramped pulses at 200 V and at a circulating temperature of 14°C using the Bio-Rad CHEF DRIII system (Bio-Rad, Richmond, CA).

Northern blot hybridization. For poly(A) RNA source, three different Northern blots were purchased from Clontech, Inc. (Palo Alto, CA): MTN human (7760-1), human II (7759-1), and human immune system (7768-1). Total RNAs were extracted by a modification of the guanidinium thiocyanate RNA purification method (Chomczynski and Sacchi, 1987) from EBV B cells and from B cells purified from tonsils by Ficoll Hypaque followed by rosetting. They were capillary blotted onto Hybond N membrane (Amersham, Arlington Heights, IL) in 20× SSC. Hybridization was performed overnight in ExpressHyb hybridization solution (8015-1) from Clontech, Inc. The membranes were washed at 5°C in 0.1× SSC and X-ray film exposed at −80°C for 1 to 14 nights.

DNA labeling. All probes were labeled by the hexamer random priming method using [α-32P]dCTP (Feinberg and Vogelstein, 1983). Probes were denatured prior to use by boiling. If the probes were not single copy, they were preannealed with 100 μg of sonicated human placental DNA for 20 min at 65°C prior to hybridization to block hybridization to repetitive elements (Tokino et al., 1991).

Cosmid, PAC, and BAC labeling. We screened three arrayed libraries; a sorted chromosome 13-specific cosmid library (Zehetner and Lehrach, 1994), a total genomic PAC library (Ioannou et al., 1994), and a total genomic BAC library (Shizuya and Lehrach, 1994), a total genomic BAC library (Ioannou et al., 1994), and a total genomic BAC library (Shizuya and Lehrach, 1994). Single-stranded end probes from each exogenous insert were generated by a linear extension from primers PL1 (5‘ ATACGACTCACTATA-GGGAG 3‘) and PL2 (5‘ ACATACGATTAGTTGACAC 3‘) of the Lawrist4 vector and from primers T7 and Sp6 of the PAC and BAC vectors. [α-32P]dCTP was directly incorporated during the synthesis, and labeled probe was hybridized directly with high-density cosmid, PAC, or BAC filters.

Fluorescence in situ hybridization analysis. An aliquot of frozen peripheral blood cells was quickly thawed, washed twice in PBS, and cultured overnight in RPMI 1640, 20% fetal calf serum. Cells were incubated with colcemid, treated with a hypotonic KCl solution, and fixed in Carnoy’s fixative. Approximately 100 to 150 ng of DNA from the cosmid clones was labeled by random priming incorporating biotin–dUTP and then treated with DNase I (Pharmacia, Piscataway, NJ) at a concentration of 50 pg/μl for 15 to 20 min at room temperature. The DNase I was inactivated at 70°C for 10 min. Approximately 150 ng of each probe in 10 μl of Hybrisol VII (Oncor, Gaithersburg, MD) was denatured at 37°C for 10 min, allowed to preanneal with human Cot 1 DNA (3 μg) at 37°C for 15 to 30 min, and hybridized overnight. The hybridization signals were detected using FITC-avidin (Vector, Burlingame, CA) and rhodamine antidigoxigenin monoclonal antibody (Boehringer Mannheim, Mannheim, Germany). Images for illustration purposes were obtained using a CCD camera (Cohu, San Diego, CA).

PCR assignment of sequence tagged sites. The sequences for the different primer pairs used in this study are available either through Genethon (Dib et al., 1996) for the microsatellites or through the Genome Data Base and the Whitehead Institute for the sequence tagged sites (STS) derived from expressed sequence tags (EST).

PCR for assignment of STSs on YAC 933E9, PACs, BACs, and cosmids from the contig was performed under conditions given in the databases. The following microsatellites were tested: D13S164, D13S1269, D13S1245, D13S1274, D13S1251, D13S1305, D13S1325, D13S1307, D13S1237, D13S165, D13S272, D13S273, AFMA-301WB5, GCT16C05, and D13S284. The different STSs tested were mapped between markers D13S153 and D13S284: WI-12824, WI-17232, WI-13561, WI-13449, WI-6333, WI-13447, WI-11681, WI-5710 (D13S912), WI-17526, WI-11665, WI-12423, WI-9598, WI-12628, SGCG3741, SGCG3580, NIB1193, WI-13764, SGCG34033, SGCG34837, SGCG3161, SGCG34145, SGCG3510, TIGR A002M11, SGCG3126, SHGC-16168, SGCG32898, A002P14, A006F33, A008Q48, WI-14979, WI-14241, A008Q05, A005R37, SGCG33707, SGCG33151, SGCG30612, and Cda11a03.

RESULTS

Mapping of YAC 933E9

A 33-member overlapping YAC contig from the Centre d’Étude du Polymorphisme Humain enabled us to order several microsatellite markers located near the D13S25 locus (Devilder et al., 1995). We have also shown that most B-CLL deletions involve a genomic area encompassing markers D13S272 and D13S294 (Devilder et al., 1995). One of the megAYACs we isolated, 933E9 (1.7 Mb), contains the markers D13S272 and D13S294 and therefore probably completely overlaps this region without significant internal deletions. This YAC is not chimeric as confirmed by FISH. Digestion of YAC 933E9 with rare-cutting restriction enzymes (NotI, MluI, and EagI), pulsed-field electrophoresis, and Southern blotting were performed. These digests were hybridized either with inter-Alu PCR probes from YAC 933E9 or with genomic fragments as probe pH2-42 for the D13S25 locus (Lalande et al., 1984) and with PCR products from the different microsatellites. Inter-Alu PCR hybridizations revealed a three-fragment (680, 350, and approximately 130 kb) MluI digest pattern. Interestingly, the 680-kb MluI fragment hybridized with all the markers in the deletion core region, i.e., D13S319, D13S272, D13S25, and D13S294. One inter-Alu PCR probe, I1Aa, was also located on this fragment. Another inter-Alu PCR probe, I1A3/4, hybridizes to the 130-kb fragment. The probe corresponding to D13S273 hybridizes to the 350-kb MluI fragment.

Contig Construction

The construction of the chromosome 13 contig was performed using threefold redundant arrayed genomic

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libraries: a chromosome 13-specific cosmids library (Zehetner and Lehrach, 1994) and total human genomic BAC and PAC libraries (Ioannou et al., 1994; Shizuya et al., 1992). The construction of the contig is outlined below, and a detailed map of the final contig is shown in Fig. 1.

Since the 680-kb MluI fragment overlaps the critical region for the deletion, we initially isolated this fragment from a pulsed-field gel and used it as a probe to screen the cosmid library. A total of 133 positive clones were identified, 45 of which were unstable/chimeric or negative when screened again with the MluI probe. Considering an average overlap of 20 kb between two contiguous cosmids and the fourfold redundancy of the library, it appeared as though the cosmids would span most if not all of the 680-kb MluI fragment.

To order the pool of cosmids, we anchored the contig at four starting points, the relative locations of which were previously determined (Devilder et al., 1995): D13S319, D13S272, D13S25, and D13S294. Screening with markers D13S319 and D13S272 detected the same cosmids, P035, G0118, I1113, and I0832, indicating that both markers are located within 40 kb of each other. All these clones contain MluI restriction sites and, therefore, correspond to the centromeric border of the 680-kb MluI fragment. Clones N0921, P035, and G0118 also contain the inter-Alu fragment IA3/4, which resides in the 130-kb MluI fragment. Therefore, this 130-kb fragment is directly adjacent to the 680-kb fragment at its centromeric side. Screening of the PAC and BAC libraries resulted in the identification of PAC 4E7 positive for D13S272 and D13S319. Hybridization with probes derived from the T7 end of PAC 4E7 identified cosmids C0422, which in turn hybridized with BAC 93D9. At the proximal side of PAC 4E7, we isolated BAC 97E4 with probe IA3/4, which overlaps the 130-kb MluI fragment as seen by hybridization to pulsed-field gel blots.

At the telomeric end of the 680-kb MluI fragment, we identified cosmids K1028, M0919, and M1743 with marker D13S294. End probes generated from M0919 hybridized with an overlapping cosmids clone N0412. Cosmid N0412 had an MluI restriction enzyme site and, therefore, contains the telomeric end of the 680-kb MluI fragment.

Screening of the PAC and BAC libraries with probe pH2-42 corresponding to the locus D13S25 resulted in the isolation of PAC 26D10. One cosmid, P0445, also hybridized to this locus. A walk from the end of K1028 detected cosmids A1124, which also hybridized to end probes from PAC 26D10, establishing a contiguous map from D13S294 to D13S25. Screening with end probes generated from the Sp6 side of PAC 26D10 resulted in the identification of cosmids A2222 and D2328. BAC 309N5, screened with an STS probe (SGC 34837) assigned to cosmids K1028 (see below), overlapped with PAC 26D10 and extended over all the telomeric cosmids.

Since we could not link the proximal and distal contigs, we chose another starting point for walking, cosmids 10550, which is contained in the 680-kb MluI fragment but not in the two existing contigs. Initially, end probes generated from cosmids 10550 identified PAC 6H3. Then, we used Sp6 end probes derived from PAC 6H3 to identify PAC 63H3. PAC 63H3 overlaps with BAC 93D9 and therefore completes the connection with the proximal contig. The distance between PAC 6H3 and PAC 26D10 was covered by overlapping cosmids, i.e., I0550, A0632, N1414, K0347, D2328, and A2222 and by the overlapping BAC 271P10.

All the cosmids isolated with the large MluI restriction fragment have been assigned to individual PACs and BACs by hybridization. The continuity between adjacent cosmids was verified using specific end probes from each clone for hybridization and confirmed using EcoRI restriction fragment digests as previously described (Ashworth et al., 1995). The length of the cloned region was estimated to be 780 kb. NotI, MluI, and EagI sites reported in Fig. 1 have been found in good concordance in YAC 933E9 and in corresponding PACs/BACs and cosmids.

Marker Assignment

Up to now, markers from different origins have been assigned to region 13q14.3 and ordered from centromere to telomere based only on linkage data, radiation hybrid analysis, and assignment to YACs. We have been able to assign by PCR some of these markers known to be in the region 13q14.3 to YAC933E9 and more precisely to PACs, BACs, and cosmids from our contig. This confirmed their relative order from centromere to telomere and provided an evaluation of the relative physical distance between each of them. We have checked markers D13S164, D13S165, D12S273, D13S1150, D13S1168, D13S272, GCT16C05, AFMA-301WB5, D13S912, SGC33510, SGC34837, D13S1269, and D13S284, which are present in the Whitehead Institute Center for Genome Research (WICGR) web site at the following address: [http://www.genome.wi.mit.edu/], and markers RB, D13S319, AFM206XF12, D13S25, D13S31, and D13S294 from the chromosome 13 workshop maps (Washington et al., 1995).

We also have checked by PCR, twice for reproducibility, new microsatellites from the Généthon map, 36 STS derived from dbEST sequences that were mapped by WICGR to the region between D13S153 and D13S284 (see WICGR web site), and two inter-Alu PCR probes (IA3/4 and IAa). Each marker assigned by PCR to the contig has also been tested by hybridization to EcoRI digests of the contig clones or to pulsed-field gel blots (digests MluI, EagI, and NotI). The summary of these experiments is shown on Fig. 1.

Markers D13S164, D13S165, and D13S284 are not present in YAC 933E9. Marker D13S273 has been located on the 350-kb MluI fragment of YAC 933E9 at the centromeric edge of our contig. Markers D13S319
**FIG. 1.** Schematic representation of the contig spanning the DBM candidate region. (A) The contig is composed of 39 cosmids, 4 BACs, and 4 PACs. The overlapping lengths between adjacent clones that are presented here are approximative. The complete cosmid names are the ones indicated, but preceded by “ICRFc108.” (B) Assignment of markers to the corresponding cosmids, PACs, and BACs. Markers corresponding to ESTs are indicated with an asterisk. (C) The minimal deletion area and the CpG island are symbolized as hatched boxes. The bold box represents the smallest common region of deletion. (D) A restriction map resulting from PFGE analysis of YAC 933E9 and corresponding cosmids, PACs, and BACs digested with NotI (N), EagI (E), and MluI (M). In the CpG island region, we could not resolve the MluI and the NotI fragments since they are very close and found on the same 4-kb EcoRI fragment.
and D13S272, which we used as starting points for the construction of our contig, are found on PAC 4E7 and are contained in the same cosmids (i.e., P035, G0118, I1113, and I0832) at the centromeric border of the 680-kb M1ul fragment. From these cosmids, we have cloned an EcoRI fragment sharing the M1ul and NotI sites and marker D13S319, but not marker D13S272. Pulsed-field electrophoresis and Southern blotting with probes corresponding to either marker D13S319 or D13S272 showed that both are on the 680-kb M1ul fragment. Therefore, marker D13S319, which is close to marker D13S272 (less than 40 kb, since they are both contained in one cosmid), can be located centromeric to this marker.

Marker AFM 206XF12 is located in PAC 4E7, cosmid C0422, and cosmid E1243. Marker AFMA301WB5 was identified in cosmids C1733, E1243, and C0422. This last cosmid overlaps the telomeric end of PAC 4E7. Therefore marker AFMA301WB5 is telomeric to AFM 206XF12, but no more than 40 kb away.

Marker GCT16C05 and marker SGC32580 are present in PAC 63H3 and in cosmid N232, which makes the connection between PAC 63H3 and PAC 6H3. An estimation of PAC 4E7, BAC 93D9, and PAC 63H3 sizes and overlappings places these markers approximately 250 kb from the centromeric M1ul site of the 680-kb fragment.

Markers D13S912 and SGC33510 and the microsatellite D13S1269 are positive on PAC 26D10. This PAC has been obtained by screening with the pH2-42 probe (locus D13S25). The likely order for these markers is, from centromeric to telomeric side, SGC33510 positive on cosmid A2222 at the centromeric end of PAC 26D10, D13S912 found on cosmid L1923, D13S1269 positive on cosmids L1923 and D1221, and D13S25 present on cosmid P0445. The microsatellite marker D13S1269, which is very close to D13S25, is much more polymorphic than probe pH2-42. Therefore, it could be a more informative marker for investigating loss of heterozygosity at this locus.

Marker D13S294 is located on cosmids M0919, K1028, and M1743. When one considers the EcoRI pattern of the cosmids between D13S25 and D13S294, this last marker is less than 100 kb distant from D13S25 at the telomeric end of the 680-kb M1ul fragment. Probe IAa does not hybridize to cosmid K1028, but does hybridize to cosmids M0919 and M1743. IAa appears to be telomeric to D13S294 and centromeric to the M1ul site. We have localized marker SGC34837 to cosmid K1028, at about 50 kb from the telomeric M1ul site of the 680-kb M1ul fragment. We also have found that D13S1150 and D13S1168 hybridize to the same 350-kb M1ul fragment as D13S273, just centromeric to our contig.

Among all the other studied STSs derived from ESTs, five of them, i.e., WI-17526, WI-12423, SCG34714, SGC34033, and Cda11a03, are also positive on YAC 933E9 but reside outside our contig. We have tested cDNA clones derived from ESTs (SGC32580, SGC33510, and SGC34837) for their expression by hybridization to total RNA extracted from EBV B cells, tonsil B cells, and poly(A)-selected RNA from a panel of 19 different tissues. SGC32580 hybridizes to a 7.5-kb RNA detected in testis, thymus, bone marrow, liver, heart, placenta, skeletal muscle, EBV B cells, and tonsil B cells. SGC34837, which contains MER 8 sequences, and SGC33510 failed to produce any distinct signal on the tested tissues after exposure.

**CpG Islands**

The number of CpG islands in the 680-kb M1ul restriction fragment was determined by digestion of the underlying cosmids from the contig with rare-cutting enzymes that contain a high proportion of CpG dinucleotides in their recognition sites. The 680-kb M1ul fragment contains at least 10 EcoRI sites: 7 in PAC 4E7, 2 in PAC 26D10 (1 in cosmids P0445 and D1221 and the other one in cosmid D1221), and 1 in cosmids O064 and K1028. Six of the 7 EcoRI sites in PAC 4E7 are found in cosmids P035. Adjacent to the centromeric M1ul site, we identified 1 NotI site, 6 EagI sites, 4 BssHII sites, and 2 NruI sites in this cosmid. A 4-kb subclone derived from the cosmids contains most of these rare restriction sites as observed on EcoRI double digests (data not shown) and is a very good CpG island candidate. The 4-kb fragment shows phylogenetic conservation using zoo blot hybridization. However, no mRNA has been detected on Northern blots of different tissues.

**Deletion Mapping**

In our previous work (Devilder et al., 1995), we have shown that in a panel of 25 B-CLL patients a deletion core encompassing D13S272 and D13S294 is formed. Using in situ hybridization we tested most of the samples described in our previous work (Devilder et al., 1995) with the PACs and BACs from the contig as probes. The results of the in situ hybridization support our earlier experiments on loss of heterozygosity in this region. Interestingly, all the samples sharing a short deletion involving D13S272 and D13S294, but not D13S273, are deleted with BAC 97E4, the most centromeric probe of the contig. Since marker D13S273 is not deleted for these patients, the centromeric breakpoint is located between D13S273 and the centromeric end of BAC 97E4. Since BAC 97E4 is estimated to be 97 kb long, the minimal deletion size for these patients must be between 780 kb and 1 Mb.

However, for one patient (No. 63), we detected a shorter deletion, since a loss of heterozygosity was observed with markers D13S25 and D13S294 only. Marker D13S272 was not deleted in this patient. We analyzed this patient further using in situ hybridization to PAC 4E7, BAC 93D9, PACs 63H3, 6H3, and 26D10. As shown in Fig. 2, monoallelic deletions were observed only with PACs 63H3, 6H3, 26D10, and BAC 93D9. However, PAC 4E7 hybridized to both chromosomes 13. Therefore the breakpoint resides somewhere
near the overlap of PAC 4E7 and BAC 93D9. From FISH analysis, cosmids C1733, C0422, and E1243 appeared deleted and cosmids I0832, I1113, G019, and H0116 not deleted. Since marker AFM206XF12 is located in this region (marker AFMA301WB5 is not informative), a PCR using the DNA of patient 63 as template was performed. No loss of heterozygosity was observed, localizing the breakpoint telomeric to cosmids G019 and H0116 and to marker AFM206XF12. To determine the location of the telomeric point of breakage, which is telomeric to D13S294, we analyzed the most telomeric cosmids and markers. As shown in Fig. 3, a junction fragment was identified with the inter-Alu probe IAa, allowing precise localization of the telomeric edge of the deletion. By pulsed-field electrophoresis and hybridization of the PACs, we estimate that the distance between the most telomeric Eagl site of PAC 4E7 and the first centromeric site of PAC 26D10 is 450 kb. Therefore, the size of the minimal region of deletion in patient 63 can be estimated to be 550 kb (Fig. 1).

**DISCUSSION**

We have shown that a 680-kb MluI fragment contains all the genetic markers contained within a minimal deletion area in B-CLL, i.e., D13S319, D13S272, D13S25, and D13S294. This fragment, which should also contain all or part of the DBM gene, is now completely covered by an overlapping 780-kb cosmid, PAC, and BAC contig. We have localized seven microsatellites (D13S319, D13S272, AFM206XF12, AFMA301WB5, GCT16C05, D13S1269, and D13S294), one restriction fragment length polymorphism (D13S25), one nonpolymorphic STS (D13S912), two inter-Alu probes (IA3/4 and IAa), and three STSs derived from ESTs (SGC32580, SGC33510, and SGC34837) at the cosmid level. These data provide information about their relative order.

**FIG. 3.** Hybridization of B-CLL DNA (digested with SspI) with inter-Alu probe IAa. H13 is a control sample derived from a chromosome 13 human - rodent monochromosomal hybrid cell line. B477 is a control sample from a normal individual. 63, 65, and 67 are DNA from B-CLL patients. T corresponds to tumor cells and B to constitutive DNA extracted from nonleukemic blood cells. The arrow indicates the junction fragment detected by inter-Alu probe IAa on patient 63.
tive positions and distances. In addition, we have de-
tected a potential CpG island located near marker
D13S319 and in a phylogenetically conserved region.
Therefore, the construction of the sequence-ready con-
tig described in this paper is an important step toward the
cloning of the DBM gene. These results also contrib-
ute to the characterization of a human chromosome 13
 genomic area located between two previously described
maps, one around RB on the centromeric side (Ford et
al., 1990) and one at the Wilson disease locus on the
telomeric side (Petrukhin et al., 1993). This contig par-
tially overlaps a recently described contig from this
region (Kalachikov et al., 1997). However, our data
show some discrepancies with the previously reported
map. For example, we show that markers AFM206-
XF12 and AFMA301WB5 are contained in the same
cosmid at less than 40 kb from each other and not at
about 80 kb, as shown in the map of Kalachikov et al.
(1997). Another point is that our estimated size of the
region is larger than the one reported by these authors.
Based upon our pulsed-field electrophoresis experi-
ments (MluI and EagI restriction sites), the distance
between markers D13S25 and D13S272 is about 490
kb, but according to the map scale presented by Kalach-
ikov et al. (1997), this distance is estimated to be 380
kb. The absence of a restriction map in their genomic
cartography may explain the differences between the
two studies. Alternatively, the characterization of YAC
745E3, which is deleted and does not contain markers
D13S272 and D13S31 as we (data not shown) and oth-
ers (Bullrich et al., 1996) have observed, may be respon-
sible for the difference. This YAC was not included in
our study because of this deletion.

In one patient, we characterized a minimal deletion
contained in the 680-kb MluI fragment that is esti-
mated to be 550 kb. Recently, Bullrich et al. (1996)
made a similar observation with a major hot spot of
deletion around marker GCT16C05. Therefore, two dif-
f erent groups assign the minimal deletion to the same
area. In this view, the CpG island located slightly cen-
tromeric to this region, the two ESTs (SGC32580 and
SGC33510) found in the deleted 550 kb, and the other
EST (SGC34837) telomeric to this region, are good can-
didates for the DBM gene.

Several authors have described different core re-
gions, however. The first descriptions (Liu et al., 1995;
Stilgenbauer et al., 1995) involve a larger area, span-
ing D13S25 and D13S273 (but excluding these mark-
ers), with a major hot spot of deletion at marker
D13S319. These data do not contradict our observa-
tions, since this core region largely overlaps the region
we define in this article. Furthermore, marker
D13S319 is not very far from markers GCT16C05 and
AFMA301WB5, which were not analyzed in the previ-
ous studies, and we believe that these results and the
ones obtained by Bullrich et al. (1996) reflect deletion
patterns that are different due to sampling, but that
involve the same tumor suppressor gene. This means
that the DBM gene spans a quite large region overlap-
ning markers D13S319 and GCT16C05 or that it is
smaller and located between these two major hot spots
of deletion. The second observation (Lichter et al., 1996)
of a minimal deletion region located telomeric to
marker D13S273 and centromeric to marker D13S272
is much more centromeric to the core region we de-
scribe. There are two potential explanations for this
result. First, several tumor suppressor genes located
in the 13q14.3 region may be involved in B-CLL. In
this case, the two core regions may reside in adjacent
tumor suppressor genes. Second, the cases described
by Lichter et al. (1996) may share deletions directly
adjacent to D13S272 that involve one end of the DBM
gene. In this case, the patient for which we described
a minimal deletion would have a deletion at the other
end of the DBM gene. The fact that most patients de-
scribed in the literature have a deleted region between
markers D13S319 and D13S25 argues, however, for an
extension of the DBM gene in this area.

Additional information is supplied by the recently
published paper of Kalachikov et al. (1997), which de-
scribes a minimal deletion delimited by two markers
they generated from the end of two clones within their
cosmid contig. The distal retained marker, 138G4/1.3R,
is located about 50 kb centromeric to GCT16C05, and
the proximal marker, 140F11-T3, is centromeric to
D13S272 according to the map of these authors. This
minimal deletion overlaps the one presented in this
article. Therefore, the smallest common deleted area
in B-CLL is bordered at its telomeric edge by marker
138G4/1.3R and at its centromeric side by the
breakpoint located between AFM206XF12 and AF-
MA301WB5. The physical distance separating these
two markers is less than 100 kb (Fig. 1). Marker
SGC32580, located close to this area, contains the 3'
end of a 7.5-kb mRNA. If it is assumed that the 5'
end extends toward the minimally deleted region, this
mRNA should be considered a strong DBM candidate.
Therefore, it is also interesting to note that the 7.5-
kb mRNA corresponding to SGC32580 is expressed in
many hematological tissues, particularly in normal B
cells extracted from tonsil. In conclusion, we report
here the shortest deletion core region for B-CLL and a
candidate mRNA close to it.

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REFERENCES

Ashworth, L. K., Alegria-Hartman, M., Burgin, M., Devlin, L., Car-


