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Molecular characterization of a new recombination of the SIL/TAL-1 locus in a child with T-cell acute lymphoblastic leukaemia

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Summary. Deletions involving the *SIL-TAL-1* locus are seen in 15% of T-acute lymphoblastic leukaemias (T-ALL). To date, seven deletions have been described, spreading over 90 kb of chromosome 1, fusing *SIL* to the *TAL-1* gene and resulting in over expression of TAL-1. During the diagnostic screening of the *TAL-1* deletion in 176 T-ALL patients, we identified one case showing a new *SIL* rearrangement. A novel fusion transcript was identified between the *SIL* exon 1a and an unknown sequence (633-cDNA). Polymerase chain reaction (PCR) screening of a human cDNA library confirmed the existence of this transcript. Using long-distance PCR on patient DNA, we obtained a genomic fragment containing *SIL* exon 1b, a portion of intron 1b, an unknown sequence and the 633 sequence. Using DNA from healthy donors, a partial genomic map of 633-DNA was found to be identical to the restriction map of the PCR fragment amplified from patient DNA. To define the chromosomal origin of 633-DNA, a YAC human genomic library was screened. Two clones containing 633-DNA were found, mapping to chromosomal region 1p32 and both contained *SIL* and *TAL-1* sequences. By searching GenBank, we identified PAC RP1-18D14 which contains *SIL*, *TAL-1* and 633-DNA, confirming this novel rearrangement as a new deletion of the *SIL/TAL-1* locus.

Keywords: T-ALL, *SIL*, *TAL-1*, PCR, breakpoint.

The *TAL-1* gene (T-cell acute leukaemia), also known as *SCL* or *TCL5*, is a transcriptionally complex locus located on chromosome 1p32, extending over 16 kb and containing eight exons, which can give rise to different mRNA transcripts by alternative splicing (Begley *et al*, 1989; Finger *et al*, 1989; Aplan *et al*, 1990a). It was originally identified in the reciprocal translocation t(1;14)(p32;q11) and is now known to be involved in *TAL-1* deletion, t(1;3)(p32;p21), t(1;7)(p32;q35) and t(1;5)(p32;q31) (Bernard *et al*, 1991; Fitzgerald *et al*, 1991; Aplan *et al*, 1992a, 1995; Francois *et al*, 1998). The *TAL-1* gene encodes at least two isoforms containing a helix-loop-helix domain (Chen *et al*, 1990a), which is found in a growing number of highly conserved DNA binding proteins involved in the growth regulation and development of all haematopoietic lineages (Visvader & Begley, 1991; Visvader *et al*, 1991; Baer, 1993; Porcher *et al*, 1996; Robb & Begley, 1996). *In vitro* and *in vivo* studies showed the existence of three different promoters: two alternative 5′ promoters, Ia and Ib (Aplan *et al*, 1990a; Lecointe *et al*, 1994; Bockamp *et al*, 1995), and a third promoter located within the fourth exon *TAL*-1 (Bernard *et al*, 1992). TAL-1 protein is expressed in erythroid, mast and megakaryocytic cells, in endothelial cells, and in the brain (Green *et al*, 1991, 1992; Hwang *et al*, 1993; Kallianpur *et al*, 1994; Leroy-Viard *et al*, 1994). Targeted gene disruption in mice shows that TAL-1 plays a pivotal role in embryonic blood formation (Shivdasani *et al*, 1995) while the oncogenic relevance of TAL-1 expression was demonstrated in studies with transgenic mice (Elwood *et al*, 1993; Robb *et al*, 1995). Moreover, the TAL-1 protein is overexpressed in the great majority of T-ALL (Bash *et al*, 1995; Chetty *et al*, 1995). Taken together, these data suggest that the dysregulation of *TAL-1* gene expression by chromosomal alterations or other unknown mechanisms may contribute to the leukaemic transformation of T-ALL.

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The *SIL* gene (SCL interrupting locus) is an early response gene discovered as the partner of *TAL-1* in *TAL-1* deletion; it is located on chromosome 1p32, spreading over 70 kb and containing 18 exons (Aplan *et al*, 1991). The function of the *SIL* gene is unclear, even though both *in vivo* and *in vitro* experiments showed that it is a highly regulated cell cycle gene, encoding for a cytosolic protein of 143 kDa. This protein does not have a significant homology with any known protein but plays a critical role in embryonic development and body axis formation (Collazo-Garcia *et al*, 1995; Izraeli *et al*, 1997, 1999).

TAL-1 gene deletion is a non-random submicroscopic deletion of 90 kb, occurring in 3–26% of T-ALL (Aplan *et al*, 1990b; Brown *et al*, 1990), but is predominantly found in childhood. As a result of this deletion, the 5′ non-coding portion of *SIL* is juxtaposed to first coding exons of *TAL-1* thus leading to an aberrant expression of TAL1 protein (Chen *et al*, 1990b; Aplan *et al*, 1992b). Different *TAL-1* deletions have been described to date (Chen *et al*, 1990a; Bernard *et al*, 1991; Aplan *et al*, 1992b; Bash *et al*, 1993; Breit *et al*, 1993a; Delabesse *et al*, 1997; van Dongen *et al*, 1999) but the two main types (1 and 2) occur most frequently and cover at least 95% of all known *TAL-1* deletions (Breit *et al*, 1993a). These deletions are mediated via recombination signal sequences (RSS) and contain randomly inserted nucleotides, both of which are hallmarks of immunoglobulin (*Ig*) and T-cell receptor (*TCR*) gene recombination processes and are restricted to T-ALL of the αβ lineage, particularly to T-ALL with *TCR* δ gene deletion on both (Akira *et al*, 1987; Hesse *et al*, 1989; Jonsson *et al*, 1991; Macintyre *et al*, 1992; Breit *et al*, 1993a).

While searching for *TAL-1* deletions by Southern blot and polymerase chain reaction (PCR) analysis, performed in 94 paediatric and 82 adult patients, we identified a putative new *TAL-1* gene deletion located 9·4 kb upstream to all the other previously described deletions.

PATIENTS AND METHODS

Patients. Bone marrow (BM) or peripheral blood (PB) samples were collected during the course of standard diagnostic procedures from 94 paediatric and 82 adult T-ALL patients referred to the Paediatric Department, University of Milano-Bicocca, Ospedale S. Gerardo, Monza, the Paediatric Onco-Haematology Unit, University of Padova, the Division of Haematology, Ospedali Riuniti di Bergamo (Italy) and the Imperial Cancer Research Fund (ICRF) Medical Oncology Unit at St. Bartholomew's Hospital, London (UK). Informed consent was obtained from patients or their legal representatives to perform laboratory analysis for diagnostic or research purposes on leukaemic cells collected at diagnosis. The diagnosis of T-ALL was established according to standard morphological, cytochemical and immunophenotypic analyses of the following panel of monoclonal antibodies: CD1a (Leu-6), CD2 (Leu-5b), CD3 (Leu-4), CD5 (Leu-1), CD7 (Leu-9), CD8 (Leu-2a), anti-HLA-DR, CD33 (leu-M9), CD34 (anti-HPCA-2), anti TcR α/β and TcR using a FACScan apparatus (Becton Dickinson, Mountain View, CA, USA). The criteria of positivity was > 30% expression.

DNA preparation and screening of patients. The purification of high-molecular-weight DNA was performed by proteinase K digestion (0·2 mg/ml) at 37°C overnight, phenol chloroform extraction and ethanol precipitation. Patients' samples were initially investigated by Southern blot analysis or by genomic PCR. A total of 10 μg of genomic DNA was digested with *Hin*dIII, *Eco*RI and *Bgl*II restriction enzymes (Bethesda Research Laboratories, BRL, Gaithersburg, MD, USA), electrophorized in a 0·8% agarose gel and then denatured, neutralized and transferred to a hybridization transfer membrane (GeneScreen Plus®; New England Nuclear, Boston, MA, USA). Prehybridization and hybridization were performed according to standard protocols using TALDB2 or SILDB probes (Breit *et al*, 1993a), whereas genomic PCR were performed as previously described with oligonucleotides kindly provided by Professor J. J. van Dongen (Rotterdam, The Netherlands).

Rapid amplification of 3′ cDNA ends (3′ RACE-PCR). 3′ RACE experiments were performed on 5 μg of total RNA purified from the patient (UPN633) and normal mononuclear cells (MNC). RNA was isolated by the guanidium isothiocyanate method (Davis *et al*, 1986). A first strand cDNA was synthesized, using a SIL sense oligonucleotide Sil exon1-5′ (5′-GCGACCCCAACGTCCCAGAG-3′) and an adaptor primer (AP) as oligonucleotide antisense. The thermal cycling was carried out for 40 cycles (30 s at 94°C, 30 s at 60°C, 1 min at 72°C). The product of the first PCR reaction was fractionated on an agarose gel purified by Geneclean kit (BIO 101; Vista, CA, USA) and amplified with a second set of PCR reactions using primers AP1 and sense oligonucleotide Sil exon1b-5′ (5′-TCCTACCTGCAAACAGACCT-3′), and the same mixture and conditions of first PCR. To exclude artefacts 3′ RACE-PCR reaction was repeated by using the Marathon™ cDNA amplification kit (Clonotech, Palo Alto, CA, USA). The final products of two amplifications were cloned into pMos vector (Amersham, Buckinghamshire, UK) and sequenced.

Long-distance PCR. A first amplification of 633 genomic DNA (633-DNA) was performed by long-distance PCR using 5′ primer Sil exon 1–5′ and 3′ primer N3R633–3′ (5′-TGCAGCCTTGATCTCCTGCAGTC-3′) designed on a new sequence identified with 3′-RACE-PCR. The purified PCR product was then amplified using sense primer Sildb (Breit *et al*, 1993a) and antisense primer N3R633 N-3′ (5′-TTTGTAGAGACAGAGCCTCCCTA-3′). The cycling protocol for both amplifications was 12 cycles of 15 s at 94°C, 30 s at 56°C, 3 min at 72°C and 20 cycles of 30 s at 94°C, 30 s at 56°C, 3 min (plus 20 s every cycle) at 72°C. Restriction enzyme analyses of the fragments obtained and subcloned were performed on 500 ng of plasmid DNA with 3–4 U/μg of restriction enzymes *Nde*I, *Eco*RI, *Bgl*II and *Pst*I. Southern blot analysis was done using probes Sb-gl and BP-NA generated by PCR amplification using primers designed on an Alu-free region. Probe Sb-gl was synthesized using-3′ oligonucleotide BP6-Z-3′ (5′-GGCAGAGGTTTTCGGGGGTCCACA-3′) and 5′ oligonucleotide BP6-V-3′ (5′-CCAAACCGGAACTGTCTGGGTCACA-3′), while probe BP-NA was obtained using oligonucleotide

sense BP-NA-5′ (5′-CAGCATACTGTTTATAAGATAAC-AT-3′) and oligonucleotide antisense BP-NA-3′ (5′-TTGG-CTTTTCATCATACTCT-3′).

Screening of a cDNA library. A commercial cDNA library synthesized from RNA of MNCs of 550 caucasian males (Clonotech) was screened with 5′ oligonucleotide 3R7–5′ (5′-CCTTCTTAGAGACTTCATAGCAGGCCAAGC-3′) and 3′ oligonucleotide N3R633–3′ (5′-GTTCAAGTCCTGTG-GACCCCCG-3′) for isoform A, and 3R6–3′ (5′-GAGA-AGGAGTCTCGCTCTATCGC-3′) for isoform B in first PCR, using the following cycling protocol: 1 min of denaturation, followed by five cycles of 30 s at 94°C and four at 70°C and 30 cycles of 30 s at 94°C and 4 min at 68°C. Nested PCR was performed using the same antisense oligonucleotides and oligonucleotide sense 3R8–5′ (5′-TTTGAGAGGGAG-GATCACTTGAGCCCAGGA-3′) for both isoforms; the cycling protocol was 5 min of initial denaturation, 40 cycles of 30 s at 94°C, 30 s of 60°C, 1 min of 72°C and 10 min at 72°C.

Screening of the YAC libraries. Screening of YAC libraries (DIBIT, Milano, Italy) was performed through successive PCR steps using 5′ sense oligonucleotide YN-5′ (5′-GCATGTGAGTCAGAAAGTTAGGTA-3′) and 3′ anti-sense oligonucleotide BP-Z-3′ (5′-GGCAGAGGTTTTCGGGG-GTCCACA-3′). Optimal conditions for the PCR reaction were determined using 20 ng of genomic MNC's DNA. The cycling protocol was 4 min of initial denaturation, followed by 30 cycles of 30 s at 94°C, 30 s at 68°C and 4 min at 68°C.

RESULTS

Screening of paediatric and adult T-ALL patients

Within a programme of study that aimed to identify new rearrangements at the *SIL-TAL-1* locus, 176 T-ALL patients (94 paediatric and 82 adult cases) were molecularly evaluated for the identification of *TAL-1* gene deletion. The presence of *TAL-1* deletions were documented in patients, using Southern blot analysis of HindIII-digested DNAs with SILDB probe or by genomic PCR analysis, using primers specifically designed for the different *TAL-1* deletion breakpoints. *TAL-1* deletion type 1 was found in 25 patients (18 children and seven adults) and type 2 in five patients (three paediatric cases and two adult cases). We did not find the other, less frequently occurring, type 3, 4 and 5 deletion breakpoints (Table I). Among the paediatric cases, the genetic lesion occurred in 21 out of 94 patients (22%), while it was found in only nine of 82 adult patients (11%) (Table II).

Table I. Frequency of TAL-1 gene deletion in T-ALL.

TAL-1 deletion	Paediatric patients ($n = 94$)	Adult patients ($n = 82$)	Total ($n = 176$)
Type 1	18	7	25
Type 2	3	2	5
Total	21 (22%)	9 (11%)	30 (17%)

Table II. Correlation between TAL-1 deletion and age.

Age (years)	Patients (*n*)	Tal-1 deletion *n* (% per age group)
0–5	30	6 (20%)
6–10	37	7 (19%)
11–15	27	8 (30%)
16–30	45	7 (16%)
> 30	37	2 (5%)

Identification of a new rearrangement involving the SIL/TAL-1 locus

Southern blot analysis enabled the identification of a patient (UPN633) carrying a rearrangement of the *SIL* gene which did not correspond to any of the known *SIL/TAL-1* rearrangements. In fact, after *Hin*d III digestion and probing with SILDB probe, patient UPN633 showed an unexpected rearranged band of about 5·5 kb, while the CEM T-cell line, used as positive control, showed a 10·5 kb rearranged band corresponding to a type-1 deletion (Fig 1). On the basis of its size, the 5·5 kb band was considered a putative new rearrangement of the *SIL* gene. Upon rehybridization of the same filter with TALDB2 probe, the *TAL-1* locus showed a germline configuration (data not shown).

Identification of a novel sequence fused to the SIL gene

By 3′ RACE-PCR experiments using total cellular RNA from patient UPN633, Sil exon 1–5′, Sil exon 1b and 3′-anchor primers, we could demonstrate the fusion between the *SIL* gene and a novel sequence (Fig 2A). Indeed, two PCR products were identified and named 633A and 633B. These two amplified products contain the Sil exon 1a, an identical 300 bp sequence and a partially different 3′ end. By searching databases, an 80% homology with Alu sequences was found in most of the 3′ end of the two cloned products. To confirm the existence of the two isoforms, a human cDNA library obtained from normal peripheral blood mononuclear cells was screened using specific primers derived from 633A (3R7 to 5′, 3R8-5′ and N3R-633-3′) and 633B (3R7-5′, 3R8-5′and 3R6-3′) (Fig 2A). The PCR

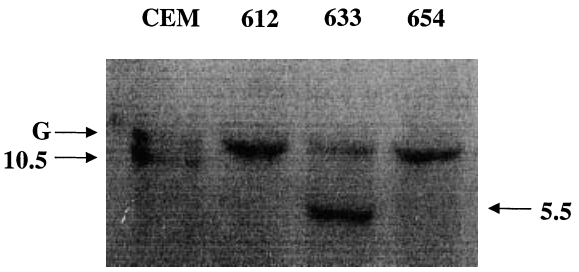

Fig 1. Southern Blot analysis of T-ALL patients and the CEM T-cell line. Genomic DNA was digested with *Hin*dIII and hybridized with ^{32}P-labelled SILDB probe. G indicates the germline configuration and arrows indicate the rearranged bands.

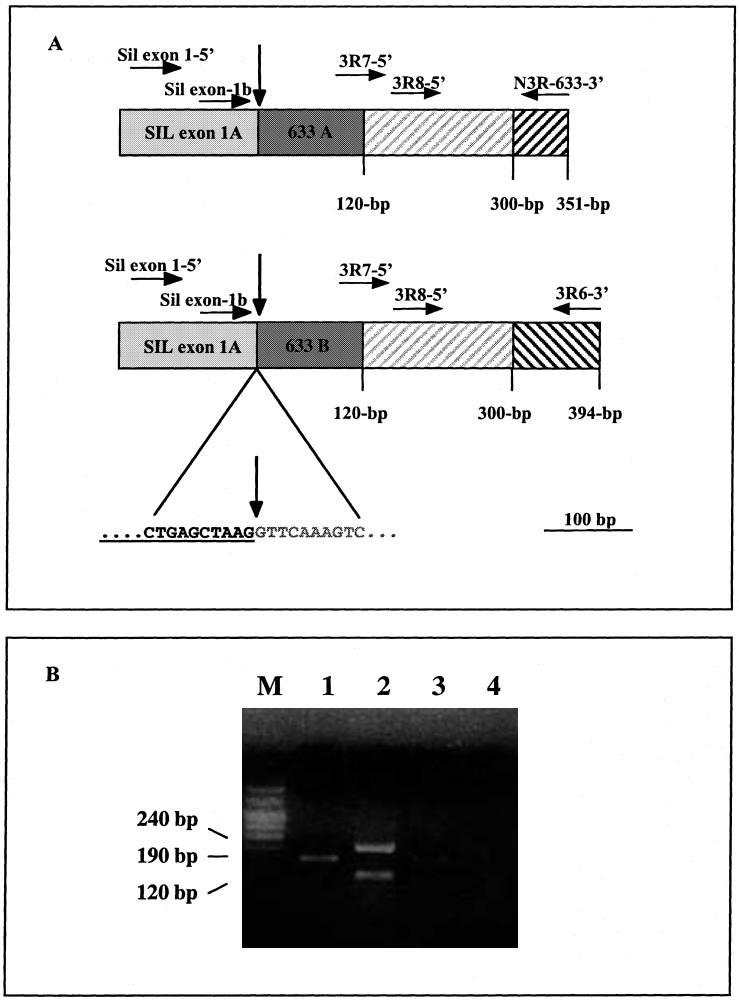

Fig 2. (A) Schematic diagram of 3′ RACE PCR products. Sil exon 1a is shown in white and the new 633 sequences fused to *SIL* are in grey. 633A (351 bp) and 633B (394 bp) sequences are identical for 300 bp (shown in pale grey) and showed 80% homology to Alu repeats, from nucleotide 120 to the end of 633-cDNA sequence (showed with hatched boxes). An arrow indicates the breakpoint. The sequence junction between SIL (underlined) and 633 is reported. (B) The amplification by semi-nested PCR (using primers 3R7–5′, N3R-633–3′ and 3R8–5′) of a commercial human cDNA library (lane1) or a 633A-containing plasmid (lane 3) enabled the amplification of the 633A sequence. When the cDNA library was amplified using primers derived from the 633B isoform (3R7–5′, 3R6–3′ and 3R8–5′), two DNA products were obtained: a 240 bp band corresponding to 633B and a 120 bp band of unknown significance (lane 2). Amplification in the absence of DNA was also performed as negative control (lane 4).

products obtained by this human cDNA library (Fig 2B) were subcloned into pMos plasmid vector, and nucleotide sequencing confirmed their identity with 633A and 633B identified in the patient UPN633 (data not shown).

Genomic characterization of the new recombination involving the SIL/TAL-1 locus

Owing to the presence of Alu repetitive sequences covering most of the 633A and B cDNAs, experiments were carried out on patient DNA to characterize the genomic breakpoint. Sense and antisense primers designed on *SIL* and 633-DNA were used in long-distance DNA-PCR amplification experiments (Fig 3A). A 5·4 kb product was obtained, and after restriction digestion with *Sac*I and *Xba*I, a 2 kb fragment was subcloned in pBluescript vector and designated Bp5. Sequence analysis revealed that the 5′ end of clone Bp5 contained exon 1b of the *SIL* gene, part of intron 1b interrupted by an unknown sequence. An oligonucleotide called BP-C-5′ was designed at the 3′ end of Bp5 and used in another long-distance PCR reaction, using the same N3R633-3′ and N3R633N-3′ antisense oligonucleotides. A 3·4 kb product was obtained which was then subcloned into pMos vector and designated Bp6 (Fig 3A). Sequence analysis showed that clone Bp6 had an unknown sequence at its 5′ end and the 633-cDNA sequence at its 3′ end. Splicing acceptor sequences were present at the junction between the unknown sequence and 633-cDNA, consisting of an intron–exon junction (Fig 3A). In order to demonstrate the presence of the 633 genomic sequence in normal DNA samples, Southern blot analyses were performed using two Alu-free probes (BP-NA and Sb-gl) located at the 5′ and 3′ ends of Bp6 (Fig 3A). Upon digestion with different restriction enzymes, the two probes identified similar fragments whose size corresponded to the restriction map obtained by long-distance PCR (Fig 3B).

Chromosomal localization of the 633-DNA sequence

As a result of the lack of informative cytogenetic data, we investigated the chromosomal localization of 633-DNA sequence by PCR screening of a human genomic DNA YAC library. Two YAC clones (810E8, 810G12) containing the 633-DNA sequence were found and both mapped at the

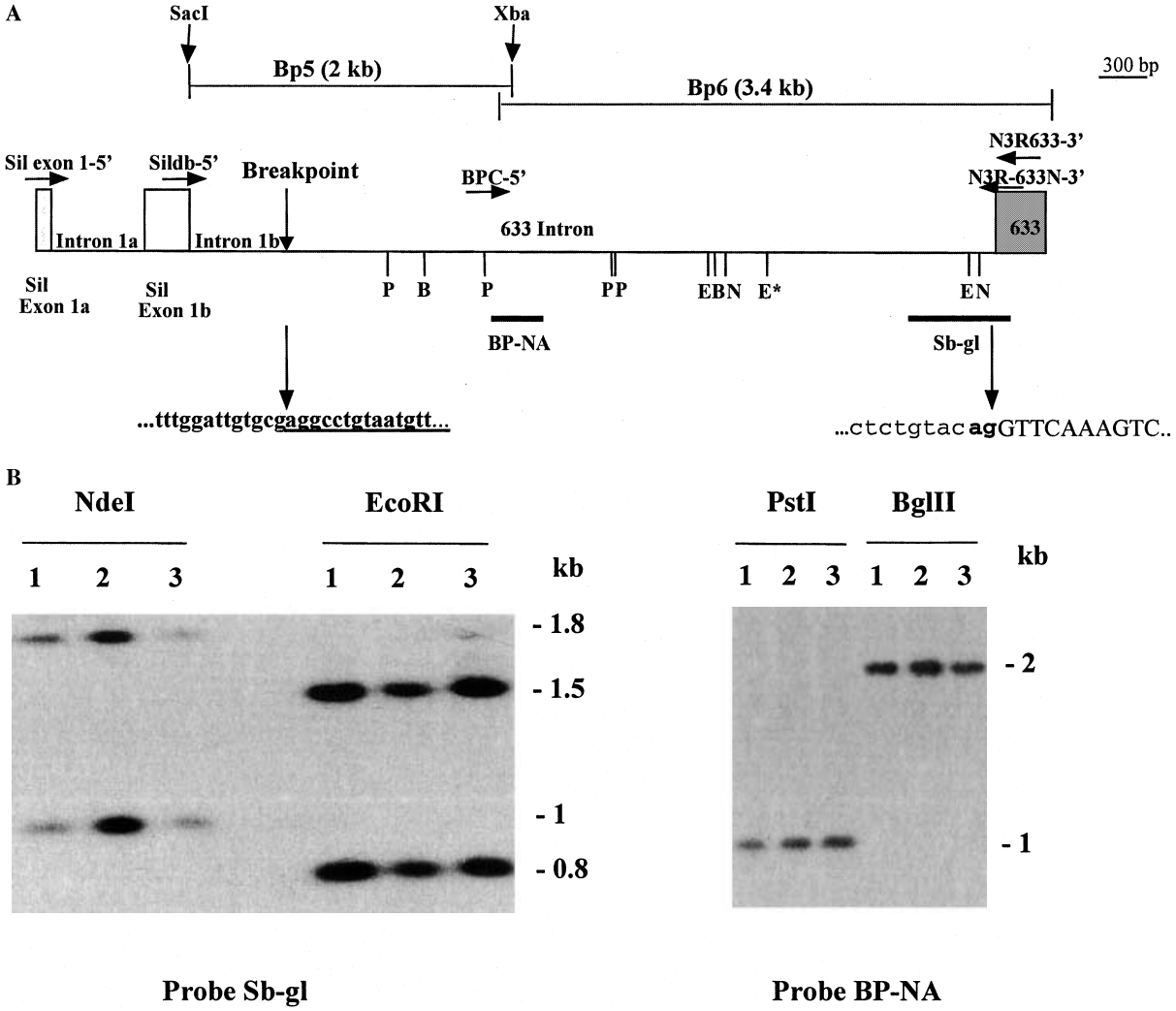

Fig 3. Schematic representation of the *SIL/633* genomic fusion (A). Genomic breakpoints and the acceptor sequence AG are indicated by arrows. *Eco*RI (E), *Nde*I (N), *Pst*I (P), *Bgl*II (B) restriction sites and probes Sb-gl and BP-NA designed on an Alu-free region and used for Southern blotting are indicated. The asterisk represents a possible polymorphic site (data not shown). Bp5 sequence was submitted to GenBank with the accession number AF415166, while Bp6 sequence with accession number AF415167. Southern blot analysis of 633 genomic region on normal DNA (B). The left panel shows digestion of genomic DNA with restriction enzymes *Nde*I and *Eco*RI, hybridized with probe Sb-gl; in the right panel, DNA was digested with restriction enzymes *Pst*I and *Bgl*II, and hybridized with probe BP-NA.

region 1p32, also encompassing the *TAL-1* and *SIL* genes. Figure 4A summarizes the dot blot results of experiments performed with a series of YACs, containing the *TAL-1* gene, and the two YACs found positive for the 633-DNA sequence. These results are in keeping with those reported in the sequence data of PAC RP1-18D14, submitted in the databank with code AL135960. Indeed, PAC RP1-18D14 contained the last exons of *SIL* (from exon 14 to exon 18), 633 sequence, *TAL-1* gene and other two genes mapped on chromosome 1p32 (Fig 4B).

DISCUSSION

A new recombination involving the *SIL/TAL-1* locus was identified in a childhood T-ALL. This finding is the result of screening for the *TAL-1* gene deletion, in a large series of paediatric and adult T-ALL. Among 176 cases, 25 were positive for type 1 and five were positive for type 2 (17%), the two main types of deletions reported to date (Chen *et al*, 1990a; Bernard *et al*, 1991; Breit *et al*, 1993a), whereas we did not find *TAL-1* deletions type 3, 4 and 5. Overall, the observed frequency of 22% and 11% in paediatric and adult cases, respectively, were in keeping with previous reports (Aplan *et al*, 1990b; Brown *et al*, 1990). We also confirmed that *TAL-1* deletions occurring in paediatric cases were associated with an immunophenotype corresponding to the mature stage of thymic maturation (Aplan *et al*, 1992b; Breit *et al*, 1993b). In the present series, T-ALL blasts from most patients carrying the *TAL-1* deletion were positive for CD3 and TcR α/β heterodimer, but were negative for CD1a,

A

YAC / Genes	810 E8	820 D11	952 E4	810 E12	762 C4	894 H7	810 G12
Si	+	-	-	-	-	-	+
Tal-1	+	-	-	-	+	+	+
633	+	-	-	-	-	-	+

B

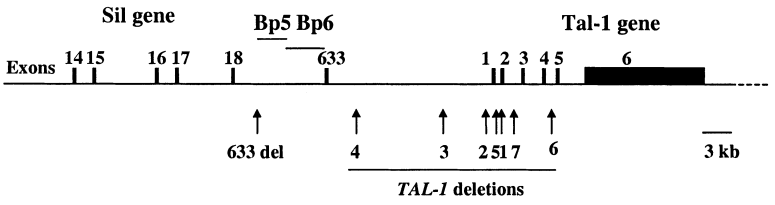

Fig 4. Mapping of the 633 genomic region. Several YAC DNA were screened for 633, *SIL* and *TAL1* genes (A). Schematic representation of the *TAL-1* region and 633 localization on PAC RP1–18D14 (B). Bp5, Bp6 and 633-cDNA are shown. The position of the 633 breakpoint and of previously described *TAL-1* deletions are also indicated (arrows).

a marker selectively expressed in the thymus cortex (data not shown).

Southern blot and PCR analyses of UPN633 supported the existence of a new rearrangement, involving the *SIL* locus with a novel partner different from *TAL-1*. This new DNA sequence did not reveal any homology with known sequences apart from ALU repeats for over 80% of its length. Sequence analysis of the fragments obtained by long-distance PCR showed a portion of the intron 1b at the end of the SIL exon 1b, followed by a new sequence containing the 633-cDNA at the 3′ end. At the junction between the known 633-cDNA and this genomic sequence (633-DNA), an intron/exon consensus boundary (CAG-GT) was found. The existence of the 633-DNA was further confirmed on digested DNA fragments from healthy donors, probed with ALU-free probes identified by BLAST (Basic Local Alignment Search Tool) database search of the 633-DNA.

At the beginning of the study, contradictory results were obtained regarding the nature of the 633 cDNA sequence. Northern blot analysis revealed the presence of a 300 bp transcript in mRNA samples isolated from T-ALL leukaemia blasts different from UPN633 and from T-ALL cell lines, by using a 226 bp probe complementary to the 5′ region of 633 cDNA (data not shown). This observation suggested the possibility that 633-cDNA could be part of a new gene. Indeed, the 3′ end of this cDNA contains three consecutive poly A signals. Moreover, both 633-cDNA isoforms A and B could be amplified from a human cDNA library. However, when PCR was performed using primers designed on isoform B sequence, in addition to the expected 293 bp band, a smaller 120 bp band was identified (Fig 2B) which failed to hybridize with an inner oligonucleotide when blotted onto a nylon filter (data not shown). Moreover, attempts to extend the 5′ end of this hypothetical novel gene with experiments of 5′ RACE-PCR failed. Similar negative results were obtained when an Alu-free probe was used to screen a human cDNA library or to perform Northern blot experiments on human mRNAs derived from different cell lines. We further expanded our sequence analysis by comparing the 633-DNA sequence with the most comprehensive *SIL/TAL-1* comparative data in vertebrates, recently published (Gottgens *et al*, 2000, 2001). Using different programmes of sequence analysis, conserved splice junctions of coding regions in the *TAL-1* locus in human, mouse and chicken have been identified. Moreover, regulatory elements (enhancer and silencer) within the boundaries of *SIL/TAL-1* genes were described (Sanchez *et al*, 1999; Sinclair *et al*, 1999; Gottgens *et al*, 2000, 2001). The ALU-free sequence of the 633-cDNA was not conserved in the mouse and no exons predicting for the 633-DNA have been identified. Therefore, we believe that the sequence initially amplified with the 3′ RACE-PCR is unlikely to represent a novel gene.

The most important advance in the definition of the new recombination came from the identification in GenBank of the PAC RP1–18D14 sequence which includes the last three exons of *SIL* (at 5′ end), the sequence identified in UPN633, and the *TAL-1* gene (at 3′ end). PAC sequence data were in agreement with our findings, supporting the conclusion that the new *SIL* rearrangement identified represents a variant of *SIL/TAL-1* deletions. This new deletion of approximately 70 kb is localized 16 kb 5′ from *TAL-1* promoter 1a, about 9 kb upstream from the breakpoint of *TAL-1* deletion type 4 (the most 5′ *TAL-1* breakpoint described to date) and 2 kb from *SIL* exon 18. Based on sequence data, we have precisely mapped the new breakpoint 54 bp downstream to Sildb3 and 479 bp upstream to Sildb2 (van Dongen *et al*, 1999).

Sequence analysis of the region surrounding the breakpoint did not reveal any similarity to RSS previously

involved in most but not all *TAL-1* deletions (Breit *et al*, 1993a). It is tempting to speculate that the ALU-rich sequence close to the breakpoint could have contributed to the genomic rearrangements, as shown in other cases (Martinelli *et al*, 2000; Blanco *et al*, 2001). The lack of frozen cells from patient UPN633 hampered any further analysis on the biological consequences of this new *SIL/TAL-1* deletion. It is likely, however, that similarly to other *SIL/TAL-1* deletions, TAL-1 protein could be overexpressed and contribute to the leukaemic transformation. The clinical relevance of identifying this or other rare *TAL1* deletions is probably negligible and only the wide application of microarray technology will enable a more precise definition of the role played by deletions on the *SIL/TAL1* locus in the induction of *TAL1* gene overexpression, and its role in determining prognosis and response to therapy.

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