High Frequency of t(12;21) in Childhood B-Lineage Acute Lymphoblastic Leukemia


The recurrent t(12;21)(p12;q22) translocation fuses two genes, TEL and AML1, that have previously been shown to be independently involved in myeloid malignant proliferations. A search for rearrangement of the TEL locus in the region known to be involved in t(12;21) was performed by Southern blotting in a panel of hematopoietic malignancies. The presence of a t(12;21) was conﬁrmed by ﬂuorescence in situ hybridization (FISH) and/or reverse transcriptase (RT)-polymerase chain reaction (PCR). We report that fusion of TEL to AML1 is speciﬁcally observed in at least 16% of the childhood B-lineage acute lymphoblastic leukemia (ALL) investigated, none of which had been previously identiﬁed as harboring t(12;21).

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THE SHORT ARM of chromosome 12 (12p) has been found to be rearranged with a variety of other chromosomal bands in various types of hematopoietic malignancies. Various 12p12 rearrangements, including partial deletions and translocations, have been reported as nonrandom abnormalities in acute lymphoblastic leukemia (ALL), and the frequency of 12p12 abnormalities was found to be as high as 10% in a series of childhood ALL. In a study of ALL patients with apparent 12p-, we have shown, using ﬂuorescence in situ hybridization (FISH) techniques, that three of eight patients studied had a t(12;21)(p12;q22) translocation not detected with conventional banding techniques. It was then shown that t(12;21) resulted in fusion of the recently identiﬁed TEL gene on 12p and the AML1 gene on 21q. Because conventional cytogenetics might miss t(12;21), we undertook systematic screening by Southern blot analysis in an attempt to evaluate the incidence of this translocation in hematologic malignancies.

PATIENTS AND METHODS

Patients. To detect t(12;21), Southern blot analysis of DNA samples from 121 patients with hematologic disorders treated at the Department of Hematology of Saint Louis Hospital in Paris (series 1) was performed. Hematologic malignancies analyzed included 54 ALLs (19 T-ALLs and 35 B-lineage ALLs; Burkitt ALLs were not included), 32 acute myeloid leukemias ([AML]s), 12 non-Hodgkin’s malignant lymphomas (NHLs), and 14 T-NHLs. There was an overrepresentation of patients with signiﬁcant leukocytosis in the peripheral blood, since patients with less than 3 × 10⁹/L leukocytes were not selected for DNA extraction. The excess of T-cell leukemia samples investigated (compared with the general incidence among ALLs) may be related to the selection of the number of circulating blast cells. AML and NHL patients in this series have been described in a previous study.

A second series of 24 cases of ALL (five T-ALLs and 19 B-lineage ALLs) investigated between January 1992 and October 1994 and 43 cases of AML (32 AML1 and 11 AML5 in the French-American-British classiﬁcation) from the Necker Hospital in Paris (series 2) was subsequently studied. These patients were also selected for the possibility to prepare DNA from leukemic cells.

Patients no. 9, 10, 11, and 12 harbored a t(12;21) and have been reported previously. All patient materials were obtained after informed consent was provided by the patients or their parents.

DNA analysis. High-molecular-weight DNA was extracted from blood samples of the patients, and Southern blots were performed according to standard procedures.

Probes used to detect the TEL rearrangement were as follows: probe A, a 1.3-kb BamHI-Aval genomic DNA fragment; probe B, a 0.4-kb HindIII genomic DNA fragment; probe B1, a 1-kb HindIII-BglIII genomic fragment, probe D, a 0.8-kb EcoRI-HindIII genomic fragment; and probe B6B7 (nts 532-1211 of TEL cDNA), a polymerase chain reaction (PCR) fragment covering mainly exon E (Fig 2).

Reverse transcriptase-PCR. Patient RNA samples were isolated after guanidine isothiocyanate disruption of the cells followed by cesium chloride selection or phenol extraction. cDNA was synthesized from 2.5 μg random primed total RNA. One-twentieth of the reaction product was submitted to PCR ampliﬁcation in 50 μL with 0.4 μmol/L primers, 100 μmol/L dNTP, and 1 U of polymerase in the buﬀer as recommended by the supplier. Primers we were as follows for TEL: B6, CACCATATAACCTCCCATC; B7, CTGGACATTTTCATAGTG; and B12, CTTGGAATCTCAACAGTCCA.5 Primes were as follows for AML1: AM3, AAGAGCTGCTACATCTTGG; and G4, CATTTCACGAGGAACGC.7-10 (Underscored nucleotides represent mutations introduced for cloning purposes.) TEL-AML1 fusion transcripts were ampliﬁed using B12 and AM3 primers in a PCR of 10 cycles (94°C × 30 minutes, 56°C × 20 minutes, and 72°C × 30 minutes) followed by 30 cycles (94°C × 30 minutes, 54°C × 15 minutes, and 72°C × 30 minutes). Primers B7 and G4 were used to amplify the reciprocal AML1-TEL fusion transcript using the same conditions, except that the annealing temperature was increased to 58°C in the ﬁrst 10 PCR cycles. Ten-μL aliquots of the PCR were then shown that t(12;21) resulted in fusion of the recently identiﬁed TEL gene on 12p and the AML1 gene on 21q.6 Because conventional cytogenetics might miss t(12;21), we undertook systematic screening by Southern blot analysis in an attempt to evaluate the incidence of this translocation in hematologic malignancies.

From U301 of the Institut National de la Sante et de la Recherche Medicales and SDI No. 15594, 1 Centre National de la Recherche Scientiﬁque (CNRS), Institut de Genetique Mol´eculaire, Paris; the Laboratoire central d’hematologie, Hôpital St Louis, Paris; and the Haematology laboratory and CNRS URA 1461, Hôpital Necker-Enfants malades, Paris, France.

Address reprint requests to O.A. Bernard, PhD, Unité 301 IN-SERM, 27 rue Juliette Dodu, 75010 Paris, France.

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Reference
cultures. RHG and/or G bands were prepared, and chromosomes were classified according to the international nomenclature.11

**FISH studies.** Unicolor and bicolor FISH analysis on metaphase and interphase nuclei and probe preparation were performed as previously described.8,12 For chromosome painting, whole chromosome 21 and whole chromosome 12 probes were used as described. The 958B8 and 93682 yeast artificial chromosomes (YACs) (isolated by screening the Centre d’Etude du Polymorphisme Humain [CEPH] YAC library with TEL-specific primers) were confirmed to give a signal on 12p12-13 on normal-metaphase chromosomes without evidence of chimerism. The 812F11 YAC, localized on 21q22.1,8,13 and 93682 YAC were used in FISH experiments on interphase nuclei. At least 100 interphase nuclei were examined in each experiment.

**RESULTS**

Detection of TEL rearrangements by Southern blot experiments. In four t(12;21) patients previously reported,6,7 the chromosome 12 breakpoints were within a single intron of the TEL gene. Taking advantage of this clustering, we decided to investigate this part of the TEL locus by Southern blot analysis of BamHI-digested DNA samples from 188 patients using a cDNA probe corresponding to this region (B6B7) (Fig 1).

An abnormal BamHI fragment was detected in four of 35 DNA samples from patients with B-lineage ALL in series 1 (patients no. 1 to 4) and in four of 11 B-lineage ALLs in series 2 (patients no. 5 to 8). Selected photographs of TEL rearrangements detected in these patients are shown in Fig 1. A genomic probe (B) lying 7.5 kb centromeric to exon E detected a distinct rearranged BamHI fragment in lanes corresponding to patients no. 1, 2, 3, 4, and 7, indicating that the chromosome 12 putative breakpoint was localized between the two probes in these patients (Fig 1).

Probe B detected the same rearranged BamHI fragment in DNA from patients no. 5 and 6, and only a germline pattern in DNA from patient no. 8.

To localize more precisely the breakpoints within the TEL intron, several probes (A, B1, D, B6B7, and E) were hybridized to Southern blots of EcoRI-digested DNA. Probe B1 detected a rearranged EcoRI fragment in five of eight DNA samples, as well as a rearranged HindIII fragment from the same patients (no. 1 to 4 and 7, Fig 2).

A t(12;21) has been previously described in patients no. 9 and 10.7 The chromosome 12 breakpoints in patient no. 9 (Fig 2) and patient no. 10 (S.P.R., unpublished results, June 1994) have been localized in the same HindIII fragment.

Taken together, the chromosome 12 breakpoints were clustered within the same 4-kb HindIII fragment in seven of 10 patients (no. 1 to 4, 7, 9, and 10). In patients no. 5 and 6, the breakpoints were localized within EcoRI fragments centromeric to the cluster (Fig 2).

The results differed in patient no. 8. Whereas probe B6B7 detected a rearrangement in BamHI-digested DNA, no rearrangement was observed with probe B. No abnormal fragments were observed in an EcoRI digest with the other probes. Since patient no. 8 was shown to have a t(12;21) (see infra), we concluded that TEL sequences centromeric to the breakpoint were deleted and that the putative breakpoint was localized in the same 6-kb EcoRI fragment as in patients no. 1 to 4 and 7 to 10.

No germline fragments were detected for patients no. 3 and 5, suggesting that loss of the normal TEL allele had occurred, as already described in other patients.6,7 Germline fragments were present in the seven other samples with TEL rearrangement.

No abnormalities of this part of the TEL locus were de-
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Fig 2. Clustering of chromosome 12 breakpoints. Hybridization was performed with the probes indicated under each photograph. Sizes of germline fragments are indicated. Note the lower intensity of germline TEL fragment in lanes corresponding to patients no. 3, 4, 5, and 9. For patients no. 3, the presence of two EcoRI polymorphic fragments, assigned to normal blood cells, indicated that deletion of the untranslocated TEL allele is somatic and not ger-}

tected in DNA samples from T-ALL, AML, or NHL patients. Overrepresentation of T-cell leukemia samples investigated (compared with the general incidence among ALLs) may be related to selection by the number of circulating blast cells.

To determine whether those TEL rearrangements correspond to t(12;21), RT-PCR and FISH experiments were undertaken.

**RT-PCR.** Transcription of both chimeric genes resulting from the t(12;21), TEL-AML1, located on der(21), and AML1-TEL, located on der(12), has been demonstrated. RNA extracted from leukemic cells bearing a rearranged TEL locus was thus tested for the presence of the corresponding chimeric transcripts by RT-PCR. Gel analysis of representative PCR experiments is shown in Fig 3A.

A specific TEL-AML1 cDNA fragment (267 bp) was amplified from seven patients tested (there was no material for patient no. 2). In the lane corresponding to patient no. 5, the amplified fragment migrated faster, similar to a minor product present in lanes corresponding to other patients. This fragment was interpreted as lacking a small AML1 intervening exon. This interpretation was confirmed by subcloning and nucleotide sequencing of this minor fragment in material from patient no. 6.

The presence of the reciprocal AML1-TEL transcript was also investigated. A PCR product of the predicted size (255 bp) was amplified from four of six samples tested (no. 1, 5, 6, and 7). No specific PCR products were seen in lanes corresponding to patients no. 4 and 8.

Three fragments were amplified from RNA of patient no. 5: one faint band of the expected size (255 bp), a major product of approximately 300 bp, and a third fragment of greater than 400 bp. To ascertain the structure of PCR products amplified from patient no. 5, major fragments obtained after both PCRs were subcloned and sequenced. They were all found to be in frame, and differed from the usual TEL-AML1 cDNAs only by transfer of the 39-bp AML1 exon from the TEL-AML1 to the AML1-TEL transcript (Fig 3B). The structure of the larger AML1-TEL fragment was not investigated.

Our data suggest that the TEL-AML1 fusion transcript, transcribed from the der(21), is crucial for the leukemogenic process.

**FISH studies.** To confirm the presence of a t(12;21) at the cytogenetic level, FISH experiments on metaphase cells were undertaken using whole chromosome 12- and 21-specific probes, or 958B8 YAC. Reciprocal t(12;21) translocations were observed in patients no. 1, 5, and 6 (Fig 4A). Only the der(21) was detected in patient no. 8, consistent with RT-PCR and Southern blotting data. Normal-metaphase cells were also present in all samples, except in patient no. 5. Patients no. 3, 4, and 7 were not studied because of shortage of material.

In the absence of material suitable for RT-PCR or metaphase FISH in patient no. 2, dual-color FISH on interphase nuclei was performed. The 812F11 and 936E2 YACs corresponding to fragments of chromosomes 21 and 12, respectively, were used as probes. Before interphase studies, the 812F11 and 936E2 YACs were tested on metaphase cells with t(12;21) (data not shown). For these experiments, 812F11 YAC was labeled with digoxigenin and the signal was revealed by anti-digoxigenin-fluorescein isothiocyanate antibodies (green signal), and 936E2 YAC was labeled with
biotin and the signal was revealed with avidin Texas red (red signal). The 812F11 YAC was asymetrically split by the t(12;21) and gave a strong green signal on the der(21) and a barely detectable signal on the der(12). The splitting of 936E2 YAC gave three red signals, one on the normal chromosome 12 and one on each rearranged chromosome. When used in FISH experiments on normal control nuclei, the two YACs gave two separate red and green signals on 97% of the nuclei of normal cells. Yellow signals, usually generated by mixing of red and green signals, were never observed in these controls.

In contrast, three categories of interphase nuclei were ob-

Fig 3. (A) Representative gel analysis of RT-PCR detection of TEL-AML1 fusion transcripts in t(12;21) ALL samples. Left: TEL-AML1 fusion cDNAs were amplified from patients with t(12;21) but not from control (HL60) cDNA. The amplified fragment in the lane corresponding to patient no. 5 is slightly shorter than usual, lacking a 39-bp AML1 exon. Right: AML1-TEL fusion cDNAs were amplified from the majority of samples tested, but not from retrotranscribed RNA from patients no. 4 and 8, control (HL60) RNA, or in the absence of template (--). In the lane corresponding to patient no. 5, the major amplified fragment has incorporated the 39-nucleotide AML1 exon lacking in the reciprocal TEL-AML1 cDNA. (B) Nucleotide sequence alignment of fused and normal TEL and AML1 cDNAs. Such alignment allowed us to redefine the boundaries of the 39-bp AML1 exon (underscored). AML1-TEL (5) and TEL-AML1 (5) are from patient no. 5. AML1A and AML1B are from Miyoshi et al and Meyers et al. TEL intronic sequences are shown as lowercase letters.

Fig 4. FISH on metaphase cell and interphase nucleus from t(12;21) patients. (A) Splitting of 958B8 YAC probe (green) on metaphase chromosomes (counterstained by propidium iodide) of patient no. 6. Three hybridization signals are seen: on normal chromosome 12 (thick arrow), der(12) (thick arrow, smaller signal), and der(21) (thin arrow). (B) FISH on interphase nucleus from patient no. 2 using 812F11 YAC normally on 21q22.1 (green, thin arrow) and 936E2 YAC normally on 12p12-13 (red). Normal chromosome 12, star; der(12), arrowhead. Superimposition of green and red corresponds to der(21), thick arrow. The nucleus is counterstained by DAPI.
served in FISH experiments performed with the same probes on nuclei from patient no. 2: 54% exhibited two red and two green signals, spread out within the nuclei, and were considered normal cells; 34% showed two red, one green, and one yellow signal, the latter of which corresponded to the superimposition of red and green signals on der(21) (Fig 4B) and was assigned to cells bearing t(12;21); and 13% showed five signals, three red and two green. One red signal, always located close to a green signal, was considered the result of splitting of the 936E2 YAC on der(21). These cells were thought to possess t(12;21).

Taken together, 47% of cell nuclei in patient no. 2 had the t(12;21) translocation.

In total, all eight patients studied demonstrated a t(12;21) by FISH and/or RT-PCR (Table 1).

Chromosome studies. Cytogenetic data concerning patients no. 1 to 8 with a proven t(12;21) are shown in Table 1. Karyotype analysis was unsuccessful in patients no. 3 and 5.

The t(12;21) per se was not detected by conventional banding techniques in any of the patients. An abnormality of 12p was seen at diagnosis in only one patient (no. 4). It was observed at relapse in patient no. 1, but was not seen at diagnosis, when the abnormality was already detectable at the molecular level.

Clinical characteristics of patients with t(12;21). Table 2 summarizes the clinical data of 12 patients with t(12;21) characterized in our laboratory6,14 (and herein). All were children (seven boys and five girls) aged 4 to 14 years (median, 8.8 years). The peripheral blood leukocyte count ranged from 3.4 to 88.7 × 10⁹/L. All patients were classified as pre-B ALL, except for Case 8, who was classified as pre-B AML. The chromosome 12 breakpoints in the four t(12;21) cases were different, with breakpoints in the long arm in Patients 1, 3, 4, and 8, and in the short arm in Patients 2 and 4.

The patients were treated with intensive multiagent chemotherapy on several different protocols, and all achieved complete remission.

Frequency of t(12;21) in hematopoietic malignancies. Southern blot screening for TEL rearrangement therefore detected eight cases in 188 patients with hematologic malignancies. In all cases, the rearrangement was demonstrated by RT-PCR or by FISH to be due to a t(12;21) translocation. Similarly, all eight cases demonstrated B-lineage ALL. All patients with t(12;21) were children, for an overall incidence of 23% in childhood B-lineage ALL (the age limit of 15 years was chosen because it is used for inclusion in the French adult ALL [LALA 94] protocol).

The t(12;21) was present in four of 35 (11%) B-lineage ALL cases in the first series and in four of 25 (16%) childhood B-lineage ALL cases. In the second series, t(12;21) was present in 21% (four of 19) of B-lineage ALL cases and reached 36% (four of 11) in children. Thus, t(12;21) occurred in 16% to 36% of childhood B-lineage ALL cases and (in none of 24 adult cases. Yates-corrected χ² test showed that the percentage of t(12;21) did not significantly differ between the two samples at the 5% level.

No rearrangements of this part of the TEL locus were detected in the other blood disorders tested (24 T-ALLs, 75 AMLs, and 35 NHLs).

Taken together, all 14 patients shown to have a t(12;21) by cytogenetics or molecular analysis6,14 (and herein) were suffering from childhood B-lineage ALL.

DISCUSSION

The t(12;21) was recently discovered during FISH analysis of ALLs with del(12)q(12),2 and was shown to result in a fusion between the TEL gene on 12p12 and the AML1 gene on 21q22.6,7 Since t(12;21) was not detected by conventional banding techniques in those cases, we decided to estimate the occurrence of t(12;21) in various hematopoietic malignancies using molecular techniques.

The chromosome 12 breakpoints in the four t(12;21) cases previously reported were within a single intron of the TEL gene.6,7 The status of this intron was thus investigated in BamHI digests of 188 DNA samples from patients with various hematopoietic malignancies. Rearrangement of the TEL locus was detected in eight B-lineage ALL patients who were selected for further characterization. The presence of t(12;21)(p12;q22) was established by FISH analysis in
five patients. RT-PCR analysis demonstrated consistent expression of the TEL-AML1 fusion transcript in all seven patients tested, whereas expression of the reciprocal AML1-TEL transcript was only found in four of six. Our data establish that the t(12;21) translocation consistently fuses TEL and AML1 genes, and indicate that the TEL-AML1 fusion transcript, expressed from the der(21) chromosome, is likely to be the important fusion transcript in leukemogenesis.

Southern blot analysis of 10 cases studied in our laboratory localized the chromosome 12 breakpoints within a 4-kb HindIII fragment in seven cases. The clustering of 70% of the breakpoints in a region that represents only 36% of the length of the intron (11 kb) is intriguing. The possibility of a common mechanism is under investigation.

Deletion of the TEL allele from the untranslocated chromosome 12 has been described in four previously characterized t(12;21) patients. In the patients reported here, TEL deletion was obvious in only two patients. In the remaining six patients, deletion of the normal allele was not found but could not be excluded, because (1) the signal of the normal allele could be derived from the presence of normal cells in samples (for example, FISH experiments showed the presence of 50% of cells lacking t(12;21) in the sample from patient no. 2), and (2) other areas of the TEL gene could be deleted that were not covered by the Southern blot analysis in this study.

Other possibilities include selection bias in the series or simple statistical variations.

Involvement of the TEL gene has been reported in a variety of rare translocations occurring in various hematologic malignancies. The TEL gene is ubiquitously expressed and encodes an ETS-related transcription factor that possesses a Helix-Loop-Helix motif at its amino-terminal end and an ETS-DNA binding domain at its carboxy-terminal end. Strikingly, the TEL gene contributes to the various chimeric products resulting from the diverse translocations by involvement of its various motifs: (1) The t(5;12)(q33;p13), seen in myelodysplastic syndrome, involves the gene for the β-chain of the platelet-derived growth factor receptor (PDGFRβ) on 5q33. Since only the TEL-PDGFRβ transcript appeared to be expressed, Golub et al suggested that in addition to ectopic expression of the PDGFRβ tyrosine kinase domain, TEL-encoded protein-protein interaction motifs activated the tyrosine kinase activity. (2) The t(9;12)(q34;p13) involving ABL on 9q34, characterized in one case of ALL, resembles the t(5;12). The ALL sample was investigated because of its deregulated ABL tyrosine kinase activity. The reciprocal ABL-TEL chimeric gene was not transcribed. (3) The t(12;22)(p13;q11), described in myelodysplastic syndrome and chronic and acute myeloid leukemias, involves the MN1 gene on 22q11, a candidate gene for meningioma, which encodes a possible transcription factor. The MN1-TEL chimeric protein, which contains the ETS-DNA binding homology domain of TEL, is likely to be the potential oncogenic result of the translocation.

With regard to the t(12;21), our data indicate that the TEL-AML1 chimeric protein is important for leukemogenesis. The predicted hybrid protein includes the 336 amino-terminal amino acids of TEL, the region of ETS proteins that is thought to interact with other proteins and to possess transactivating properties, and almost all the AML1 product. Thus, the TEL gene contributes not only promoters but also the protein-protein interaction domain to the DNA binding and transactivation motifs encoded by AML1. It has been claimed that an additional characteristic of the t(12;21) is the loss of the other TEL allele, obvious in some cases. Several explanations could account for this deletion. It could reflect the loss of a tumor suppressor gene, potentially TEL. Alternatively, the TEL-AML1 fusion product resulting from t(12;21) could act in a recessive fashion and thus requires deletion of the normal TEL allele.

Functional studies are needed to understand the exact contribution of TEL in the various fusion proteins resulting from these translocations.

The t(12;21) was specifically observed in childhood B-lineage ALL with a frequency of 16% in one series and 36% in a second. This discrepancy is not statistically significant and could be due to the smaller number of patients studied in series 2 (11 v 24 children). However, our data indicated that t(12;21) is the most frequent translocation in childhood leukemia.
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B-lineage ALL. Systematic detection of t(12;21) in this population will provide a more accurate frequency.

No TEL rearrangements within the regions investigated were detected in the other hematopoietic malignancies, including AML, T-ALL, and NHL. However, we have only investigated a restricted area of the TEL locus, thought to encompass greater than 150 kb.\(^{15,17}\) Putative variant t(12;21) translocations and other types of translocations such as t(12;22) would therefore have been missed in our investigation.

Cytogenetic studies of patients bearing t(12;21) confirmed that it was not detectable by classic cytogenetic techniques. Among t(12;21) patients detected by Southern blotting, only one patient (no. 4) had exhibited a 12p abnormality at diagnosis. It is therefore highly unlikely that t(12;21) accounts for all the 12p abnormalities observed in 10% of ALLs.\(^{3}\) The question of how many genes are involved in these abnormalities needs to be addressed.

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