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# Prenatal Origin of *TEL-AML1*–Positive Acute Lymphoblastic Leukemia in Children Born in California

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Acute lymphoblastic leukemia (ALL) is the most common form of childhood cancer. The peak incidence of ALL between ages 2 and 5 is accounted for by one subtype, referred to as common acute lymphoblastic leukemia (cALL). About 25% of cALL patients have the *TEL-AML1* gene fusion derived from the t(12;21) chromosomal translocation. Recent evidence from retrospective analysis of neonatal blood spots (Guthrie cards) in Europe has demonstrated that this chromosome translocation may arise prenatally. The aim of our study was to determine whether *TEL-AML1* fusions arise prenatally in a U.S. population of cALL patients. *TEL-AML1*–positive cALL cases (n = 14) were identified by fluorescence in situ hybridization, and the genomic breakpoints were identified by a streamlined long-distance PCR approach and sequenced. Clonotypic primers were designed for each patient breakpoint, and a nested PCR assay was used to determine the presence of the *TEL-AML1* fusion sequence in neonatal Guthrie cards. Seven of 14 cases demonstrated clonotypic sequences on the archival Guthrie cards. The oldest patient that was positive was 6.7 years old at the time of diagnosis of leukemia. These results confirm previously published findings of a prenatal origin of *TEL-AML1* in Europe by demonstrating its occurrence in a California-born population. Secondary changes were also similar to those described previously, with deletion of the second *TEL* allele being the most common. Other secondary changes included duplication of the fusion gene, trisomy 21, and monosomy X.
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## INTRODUCTION

As with other types of cancer, leukemia is a heterogeneous disease whereby nonrandom genetic aberrations, such as chromosomal translocations, are complemented by secondary genetic aberrations that give rise to leukemic clones. In infant leukemia and common acute lymphoblastic leukemia (cALL) of early childhood, all of the molecular changes necessary to generate disease occur within a relatively narrow time frame, affording an opportunity for investigation of the etiology of such leukemias.

Compelling evidence has demonstrated that chromosomal translocations are early events in leukemogenesis, occurring in utero. Initially, studies in twins with concordant leukemia demonstrated an in utero origin for infant leukemia characterized by *MLL* rearrangements (Ford et al., 1993; Gill Super et al., 1994) and cALL characterized by t(12;21) *TEL-AML1* rearrangements (Ford et al., 1998; Wiemels et al., 1999b). More direct evidence for an in utero origin of specific translocations was provided by retrospective scrutiny of neonatal Guthrie blood spots for the presence of patient-specific chromosomal translocations, including *MLL* (Gale et al., 1997) and *TEL-AML1* (Wiemels et al., 1999a; Maia et al., 2001). Indirect support for a prenatal origin of several ALL cases lacking specific chromosomal translocations was provided by tracking clonotypic *IGH* and *TCR* rearrangements including cALL (Yagi et al., 2000), T-ALL (Fasching et al., 2000), and hyperdiploid ALL (Taub et al., 2002).

The *TEL-AML1* rearrangement is observed in about 25% of precursor B-cell ALL, in children diagnosed between the ages of 2 and 10 years (Romana et al., 1995b; Shurtleff et al., 1995;

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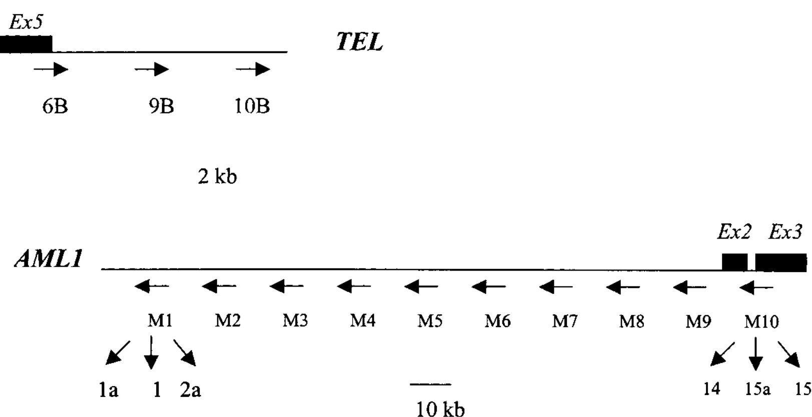



Figure 1. *TEL* and *AML1* long-distance primer design. Primers used for long-distance PCR are represented schematically for the *TEL* and *AML1* genes. Primers sequences are listed in Table 1 (*TEL*) and Table 2 (*AML1*). For *AML1*, the multiplex groups are shown and the three primer members of groups 1 and 10 are shown to illustrate the orientation. *TEL* intron 5 is about 14.5 kb (GenBank accession nos. U61375 and U63313) and *AML1* intron 1 is about 160 kb (GenBank accession no. AP001721.1).

Borkhardt et al., 1997). As described above, it has been demonstrated to arise prenatally in several studies based in Europe (Ford et al., 1998; Wiemels et al., 1999a; Maia et al., 2001), with the oldest patient recorded as age 14 years (Wiemels et al., 1999b). The aim of this study was to confirm these findings in a population of patients with cALL in California.

## MATERIALS AND METHODS

### Patients

Diagnostic samples (n = 14) were obtained from a cohort of patients enrolled in the ongoing Northern California Childhood Leukemia Study (NCCLS), previously identified as *TEL-AML1*-positive by fluorescence in situ hybridization (FISH) (see below). The patients ranged in age from 2.2 to 6.7 years. Corresponding Guthrie cards (neonatal blood spots) for each patient were obtained from a central repository maintained by the Genetic Diseases Branch of the California Department of Health Services.

### Interphase FISH Detection of t(12;21) (*TEL-AML1*)

The FISH method applied in this study was designed to detect t(12;21) and hyperdiploidy (>50 chromosomes) simultaneously. The LSI *TEL-AML1* and alpha-satellite CEP X probes were purchased from Vysis (Downers Grove, IL). The *TEL* probe begins between exons 3–5 and extends approximately 350 kb toward the telomere on chromosome 12 and is labeled directly with SpectrumGreen. The *AML1* probe labeled directly with SpectrumOrange spans the entire gene of approximately 500 kb. The centromere probe of the X chromosome is labeled with SpectrumAqua. The hybridization procedure was followed according to the company's protocol. FISH signals were viewed with a triple-band filter (Chroma Technology, Brattleboro, VT). The different clone cell types were observed and confirmed by two experienced cytogeneticists (at least 100 cells scored).

### Preparation of DNA

High molecular weight DNA was isolated from the cryopreserved peripheral blood or bone marrow lymphocytes of diagnostic samples, depending on the availability, by use of standard SDS/Proteinase K protocols (Sambrook et al., 1989).

### Amplification of Genomic Breakpoints by Long-Distance Multiplex PCR

A long-distance multiplex protocol was designed to amplify the *TEL-AML1* breakpoints from patient DNA samples. Three primers from *TEL* intron 5 (6B, 9B, 10B) were used in a long-distance PCR reaction in combination with 30 primers (spaced approximately 5 kb apart) from *AML1* introns 1 and 2, to cover the breakpoint region in portions amenable to amplification (Fig. 1, Table 1). For each sample, 10 multiplex reactions were set up as an initial screening method, with each PCR reaction containing the three *TEL* primers in combination with a single multiplex group of three *AML1* primers (Fig. 1). Primers were designed to generate products under standardized amplification conditions and to exclude interference with other primers in the multiplex group, such as primer–dimer formation. When a positive product was obtained, nine individual reactions were performed by use of each *TEL* primer in combination with one of the three primers within the *AML1* multiplex group to identify which pair of primers had generated the product. PCR products were then purified from agarose gels by use of the QIAquick PCR Purification kit (Qiagen, Chatsworth, CA) and se-

TABLE 1. *TEL* and *AML1* Primers for Long-Distance PCR

| Primer name | *TEL/AML1* | Sequence[a] 5′–3′ | Multiplex group |
|---|---|---|---|
| 6B | *TEL* | CTCTCATCGGGAAGACCTGGCTTACATG | N/A |
| 9B | | GTCAGTGTGCTTGGAATCATAGGTGC | |
| 10B | | CCAGGTGAGCCGTGGCAACAAC | |
| 1a | *AML1* | CCTCCTGTAATGTCCTCCAGGGCA | M1 |
| 1 | | GGACTCTGGCACCAACCAGCTATGG | |
| 2a | | AAGCATGGAGAAGTGACCTCCCAGA | |
| 2 | *AML1* | GCAAGCAGTTACTTTCCTGGTCTGCCA | M2 |
| 3a | | TCAGACTGAGGCTGCAGCTCATGGCA | |
| 3 | | GGAAGGGCTGGTAGCTTTGGGTCC | |
| 4a | *AML1* | ACGGACTTCAGTTTGCTGAGCCCTGA | M3 |
| 4 | | CTTGGCACCAATGTCCTCTTCACTCC | |
| 5a | | CTCCTGAGTCAGACATTAGCATGGCT | |
| 5 | *AML1* | GCAGTAGGAGATGGTGTGTGGAACACC | M4 |
| 6a | | GGGTGGGCTCTGAATCTAATGACTGA | |
| 6 | | CCTTCAGAGGGAGCACAGAATGCC | |
| 7a | *AML1* | GGACCTATTCTAGAAGCCTGGTGCCA | M5 |
| 7 | | CGAACACCGTGGAAGCTCTCTGATGT | |
| 8a | | ATACAGGCCTAACTACACCAGAAGGT | |
| 8 | *AML1* | GGTTTACTGCCCTGCTGTCCAGAGG | M6 |
| 9a | | CCCTTATGGTGCTGGCTGTGACCGA | |
| 9 | | CAGATGAAGGAGAAGGTTGCTGTCCTAACA | |
| 10a | *AML1* | GAGGCCGAGGCAGGTGGATCACT | M7 |
| 10 | | ACCATCCTTTGACCTTGGCCTTGC | |
| 11a | | CAGGTTAGCCCAACGATTGCGCAACA | |
| 11 | *AML1* | GAGGCCATGAGGGTGTGAATCAGG | M8 |
| 12a | | AGAGTACATCCAGGGAGGACGCTGA | |
| 12 | | CTACAAGGTGTAGAGGTGGCACAGGGTG | |
| 13a | *AML1* | TCTGGTGACCAGTGAGAACCAAGACA | M9 |
| 13 | | CTCTCTCAGTCAGTGTGAAGCAGGAGCC | |
| 14a | | TGCTTCTGAAGGGCAGCCACTCTCCA | |
| 14 | *AML1* | CCAAGTGGAGCATCTGAAAATTGCGC | M10 |
| 15a | | GAGCCTGCACTTTCAAGGACAGCCA | |
| 15 | | AACGCCTCGCTCATCTTGCCTGG | |

[a]Sequences for each *AML1* multiplex group, illustrated in Figure 1, are listed.

quenced by use of forward and reverse PCR primers. Further primers were designed as necessary to "walk in" to the breakpoint sequence.

Long-distance PCR was performed by use of eLONGase Enzyme mix (Life Technologies, Gaithersburg, MD) according to the manufacturer's protocol. As recommended, a two-step amplification was performed with an initial denaturation at 94°C for 30 sec, followed by 35 cycles of denaturation at 94°C for 30 sec and annealing/extension at 68°C for 20 min. A $MgCl_2$ concentration of 1.7 mM was used in all reactions.

For cases where limited sample material precluded the long-distance PCR approach described, a previously published inverse long-distance protocol was used to obtain patient breakpoints (Wiemels and Greaves, 1999).

### Guthrie Card Analysis

A clonotypic nested PCR assay was designed for each patient breakpoint. Assay specificity was confirmed by testing with patient-specific DNA as well as DNA from several other patients (each with a unique breakpoint). Assay sensitivity was deter-

TABLE 2. *TEL-AML1* Fusion Sequences for Each Patient*

| Patient ID | Translocation fusion sequence (der12) | *TEL* breakpoint[a,b] | *AML1* breakpoint[c] |
|---|---|---|---|
| P1 | GTGATTCCTGTGTACAACGTACC**TA**CAAACCATGAAAAAC | 31,545[a] | 233,742 |
| P2 | TGTGAATTCCCAGGCTGCA**C**TGGTAATAGACGCAGGTGGC | 36,531[a] | 164,295 |
| P3 | CTGGAGGGTATAGATGGA**AA**GTTAGGAGTGGGTTCGTAGA | 31,722[a] | 200,745 |
| P4 | GTTCCGAAAAGGCTGAAAtggcATTGCAATCAGGAAACCA | 38,006[a] | 161,105 |
| P5 | TGGAGGTGAAATTCATTG**GG**AAGCCAAGTCCACTGAGGAA | 31,526[a] | 238,616 |
| P6 | TGCACCACCAGTGTTGTCTCTCTATCACTGTGAGCACACA | 29,685[a] | 195,137 |
| P7 | TTCCCACTTCATAAGTGAGG**CA**CTGTCTTAAACACACGCA | 2,766[b] | 147,578 |
| P8 | TTTCCTAGGATGCTCAT**AAT**GTCAAACTGTATG**TT**ATATA | 28,723[a] | 254,305 |
| P9 | CTACTGTCTGCCAGAGTAATACTAGGAGATTGTATCCGTCC | 29,024[a] | 229,591 |
| P10 | CTGGCCTCAAGTGATCCACCATTTTTAGAGTCTCCTAAGC | 3,597[b] | 166,809 |
| P11 | CCCCTTTCAAATGTGCCAGGCGGAGGCTGAGGCTGGGGCA | 2,932[b] | 155,718 |
| P12 | TTACCAGGTTGGAATGGCTAACATTTTAGAGATTCTGGTT | 33,363[a] | 243,221 |
| P13 | AATTGTTCTCACTCATAGGTcccCAGACTACTTCAGAGTC | 32,928[a] | 289,466 |
| P14 | ATGAAAGAGCTGAGCACAG**T**GCTGAGTGGGAAGCCATGAT | 31,915[a] | 281,506 |

**TEL* sequences are underlined. Microhomologies (nucleotides common to both *TEL* and *AML1*) are noted in bold. Non-template *N* nucleotides are denoted in lowercase.

Locations of breakpoints are referenced to GenBank sequences. *TEL* intron 5 is covered by two accessions:

[a]GenBank accession U61375 and

[b]GenBank accession U63313.

[c]*AML1* introns 1 and 2 are covered by a single accession: GenBank accession AP001721.

mined by use of serial dilutions of patient-specific DNA, and in some cases semi-nested reactions showed greater sensitivity.

Amplification was performed with Ampdirect buffers (Rockland Immunochemicals, Gilbertsville, PA), according to the manufacturer's protocol. ELONGase enzyme (Life Technologies) was included in the reactions. Before scrutiny of Guthrie cards for clonotypic sequences, each Guthrie card was tested for DNA quality and amplifiability with primers for NAD(P)H:quinone oxidoreductase (NQO1) in a single-round PCR amplification. Primer sequences and PCR conditions were as previously published (Wiemels et al., 1999a).

For analysis of Guthrie cards, 1/16th pieces were placed directly in first-round PCR reactions (50 μL), or 1/8th pieces were soaked twice in 500 μL sterile PCR-grade water and vacuum dried before addition to the PCR reaction. Second-round amplification (25 μL) was performed with 1 μL from the first-round PCR reactions. PCR conditions included two pre-incubation steps of 80°C for 15 min and 94°C for 4 min, followed by 40 amplification cycles of 94°C for 30 sec, 58°C for 1 min, 72°C for 1 min, and, finally, a 72°C extension step for 7 min. For each patient, at least three quarters of a 1.5-cm spot were analyzed. Serial dilutions of patient diagnostic DNA were analyzed as positive controls were. Negative controls included water and card pieces from a normal non-affected individual.

## RESULTS

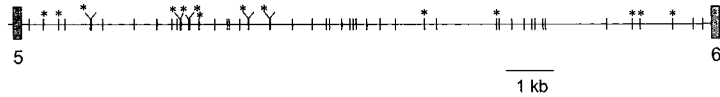
### Detection and Characterization of *TEL-AML1* Fusion Sequences

Interphase FISH detected *TEL-AML1* fusion gene in 14 patients from the NCCLS, ranging in age from 2.2 to 6.7 years. *TEL-AML1* breakpoints from these 14 cALL cases were identified by multiplex long-distance PCR and sequenced. Table 2 shows the fusion sequences and intronic locations of the breakpoints in the *TEL* and *AML1* introns. The distribution of the breakpoints was similar to earlier reports, with an apparent cluster of four breakpoints within a 390-bp region of *TEL* and with a much wider distribution of breakpoints in the large *AML1* intron (Thandla et al., 1999; Wiemels et al., 2000) (Fig. 2). Seven of 14 sequences exhibited microhomologies of between 1 and 3 nucleotides. Two (P4 and P13) of the 14 fusion sequences described had non-template *N* nucleotides (Table 2), in agreement with sequences reported previously (Thandla et al., 1999), although absence of these nucleotides was reported in another study (Wiemels et al., 2000).

### Analysis of Guthrie Cards

Nested or semi-nested PCR assays were established for each clonotypic sequence and the corresponding Guthrie cards analyzed for the presence of these sequences. Segments were tested in two different ways. Initially for cases P6, P7, and P8,

TEL




AML1

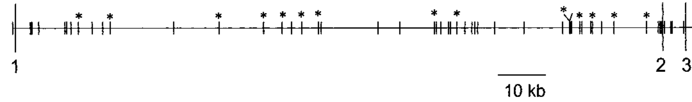



Figure 2. Distribution of 52 translocation breakpoints along *TEL* intron 5 and *AML1* introns 1 and 2. Breakpoints from individual patients are noted to scale by hash marks. Exons are noted by numbers. Fourteen breakpoints from the current study are noted with asterisks. Additional breakpoints are derived from previous publications [Romana et al. (1999), #34; Wiemels and Greaves (1999), #18; Wiemels et al. (2000), #17; Ford et al. (1998), #3; Maia et al. (2001), #7; Andersen (2001), #33].

TABLE 3. Characteristics and Backtracking Results Among 14 Newly Diagnosed Cases of Leukemia in Children* Born in California

| Patient ID | Age at diagnosis | PCR sensitivity[a] (pg) | No. Guthrie segments tested | No. Guthrie segments positive for *TEL/AML* |
|---|---|---|---|---|
| P1 | 2.9 | 10 | 14 (1/16th) | 3 |
| P2 | 3.5 | 10 | 14 (1/16th) | 1 |
| P3 | 4.7 | 100 | 12 (1/16th) | 0 |
| P4 | 2.2 | 10 | 12 (1/16th) | 0 |
| P5 | 3.2 | 100 | 12 (1/16th) | 0 |
| P6 | 4.7 | 100 | 6 (1/8th) | 0 |
| P7 | 2.8 | 10 | 6 (1/8th) | 2 |
| P8 | 4.0 | 10 | 6 (1/8th) | 1 |
| P9 | 4.6 | 10 | 12 (1/16th) | 0 |
| P10 | 4.3 | 10 | 12 (1/16th) | 2 |
| P11 | 2.3 | 10 | 12 (1/16th) | 4–6 |
| P12 | 6.7 | 10 | 12 (1/16th) | 2 |
| P13 | 2.5 | 100 | 12 (1/16th) | 0 |
| P14 | 2.4 | 10 | 14 (1/16th) | 0 |

*The number of segments positive for clonotypic *TEL-AML1* for each of the 14 cases tested is shown.
[a]The PCR sensitivity is shown as pg of diagnostic DNA detectable in PCR assay (10 pg is approximately 1–2 cells, and each Guthrie card segment contains about 4,000 cells; thus sensitivity is in excess of 1 in 1,000).

1/8th segments were soaked twice in distilled water and vacuum dried before addition to the PCR. For the remaining samples, 1/16th segments were added directly to the PCR because of concerns of DNA leaching out during the washing steps. For each sample, the equivalent of at least 12 × 1/16th segments were tested in two separate assays along with patient-specific DNA positive controls, a control card containing DNA from a normal non-affected individual, and PCR water negative controls.

Seven of the 14 cards tested had amplifiable *TEL-AML1* fusion DNA in at least one segment (Table 3, Fig. 3). Figure 3 shows the PCR result for each positively backtracked sample. Guthrie spots were tested for each case in two separate assays (Table 3 shows the total number of segments tested per patient). Prenatal *TEL-AML1* was detected in P1 in two separate assays, two positive segments of eight tested are shown here, and, in a separate assay (not shown), one of six segments was positive.

The ability to detect clonotypic sequences on Guthrie segments requires both a sensitive assay and the presence of the target (if present) at sufficient levels so as to be detectable by the assay. Table 3 shows each backtracking result correlated with the sensitivity of each corresponding PCR assay. For all seven positive cases and three of the negative cases, a sensitivity of 10 pg of diagnostic DNA was attained (Table 3), which is close to detection at the single-cell level (a single lymphocyte contains ~6 pg DNA). The remaining four negative cases (P3, P5, P6, P13) had assay sensitivities of 100 pg. Sensitivity of the PCR assay is determined in part by primer design, which in turn is constrained by the need to generate small products to avoid problems with degradation of archival samples. Although sensitivity of the PCR assay may be one factor in the four negative cases with

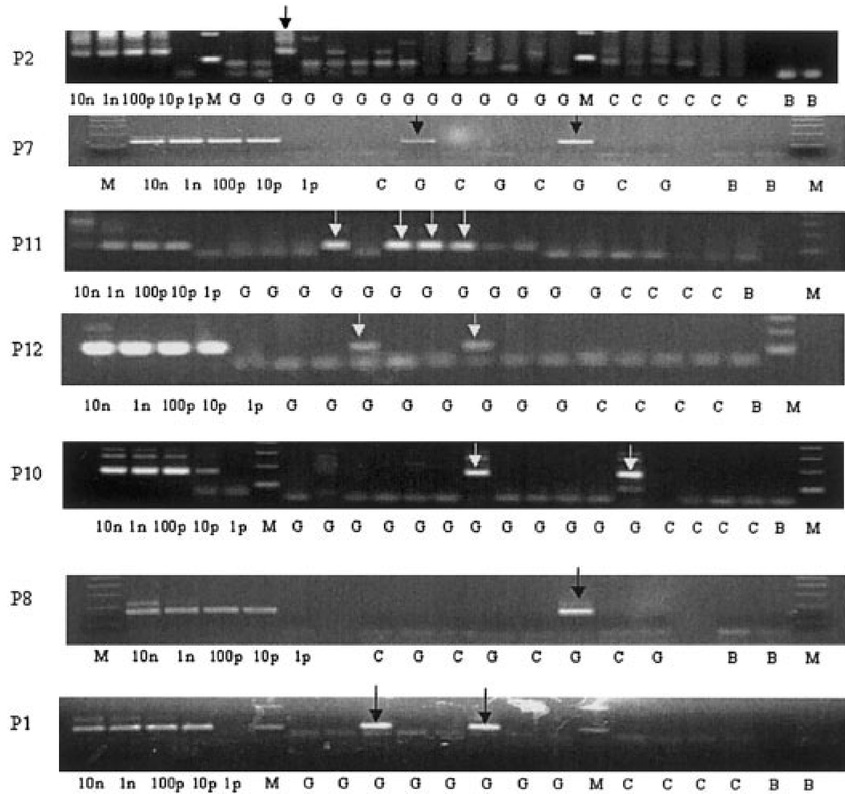



Figure 3. Positive backtracking PCR results. The figure shows the results of the clonotypic PCR assay for each of the seven positively backtracked cases after agarose gel electrophoresis. Standards are shown on the left, ranging from 10 ng to 1 pg of diagnostic DNA. M, molecular weight marker; in most lanes, the 100- and 200-bp bands are visible. G, Guthrie segments; C, control cards; B, PCR water blank control.

sensitivity of 100 pg, all seven negative cases should be considered uninformative.

## DISCUSSION

The findings reported here confirm two previous European backtracking studies that used Guthrie cards, which concluded that the *TEL-AML1* translocation often occurs prenatally and may be an initiating event in cALL (Wiemels et al., 1999a; Maia et al., 2001). One study reported 11 cases, including a pair of twins, of which eight had detectable *TEL-AML1* fusion (Wiemels 1999a), whereas the second study reported a set of triplets with identical prenatal *TEL-AML1* fusion sequences (Maia et al., 2001). In the present study, clonotypic sequences from 7/14 cases were demonstrated to have an in utero origin, the youngest patient being 2.3 years old and the oldest 6.7 years old. This is the oldest reported cALL case to have been positive by this direct type of assay, although a 14-year latency period was previously reported in a twin case in which the clonotypic sequence was retrospectively detected in a sample obtained 9 years earlier when the first twin was diagnosed with leukemia (Wiemels et al., 1999b). The remaining seven cases, which had negative Guthrie spot PCR results, have to be considered uninformative, given that several factors could have influenced the result, including inadequate PCR sensitivity, subthreshold numbers of pre-leukemic cells in blood at birth than are detectable on the limited Guthrie specimen, and differences in the quality of DNA on the Guthrie cards.

In this study, a multiplex long-distance PCR protocol was used to obtain breakpoint sequences. This approach was designed to amplify regions of not more than 10 kb and is a faster, more streamlined approach than the previously used method of inverse PCR (Wiemels and Greaves, 1999) because it allows the approximate location of the breakpoint to be ascertained in one day, whereas the prior inverse PCR approach takes 4 days and results in a higher rate of false positives. It may also be a useful method for minimal residual disease detection because the clonotypic nature of the sequences precludes cross-contamination from other patient samples. However, the inverse PCR approach is the only feasible method when diagnostic material is limiting.

The characteristics of the individual breakpoints reported here are similar to previous findings. Wiemels et al. (2000) reported microclustering of

breakpoints in the *TEL* and *AML1* genes based on analysis of 24 sequences. The 14 novel fusion sequences reported here are found distributed across the respective introns in a similar fashion (Fig. 2), and do not further refine microclusters identified previously. Only two of the 14 sequences have non-template N-nucleotides at the chimeric junctions, also observed in three previously reported sequences (Thandla et al., 1999), but absent from a further 20 sequences (Wiemels et al., 2000). The presence of these N-nucleotides alone is not sufficient evidence of V(D)J recombination. The suggested mechanism of translocation generation is error-prone non-homologous end-joining repair of double-stranded breaks in DNA (Chu, 1997).

The concordance rate for childhood ALL in identical twins is around 5% (Ford et al., 1998), indicating the requirement for secondary postnatal events. This suggests that considerably more children are likely to acquire a *TEL-AML1* fusion in utero than ever have a diagnosis of ALL. Indeed, from 567 newborn cord blood samples screened for t(12;21), about 1% had a *TEL-AML1* fusion gene (Mori et al., 2002). One percent represents 100 times the cumulative rate of risk of ALL with *TEL-AML1*, indicating that the frequency of conversion of the pre-leukemic clone to overt disease is low. As previously discussed, post-natal exposures to chemicals or electromagnetic radiation, additional chromosomal or molecular abnormalities, genetic susceptibility, or infection may all help to promote progression to leukemia (Greaves, 1999, 2002).

Secondary changes at the chromosome level include deletion of the non-rearranged *TEL* allele and trisomy 21. Initial reports showed that the non-translocated allele of *TEL* was deleted in each of four cases (Golub et al., 1995; Romana et al., 1995a). In a more recent study, microsatellite mapping of the 12p deletions in a pair of monozygotic twins sharing a clonotypic *TEL-AML1* sequence demonstrated the *TEL* deletions to be different and therefore most likely independent events occurring after birth (Maia et al., 2001). Other studies of *TEL* deletions by FISH in singleton patients with ALL suggest that this genetic abnormality is frequently restricted to a subclone of leukemic cells and is therefore most likely to occur as a secondary event (Raynaud et al., 1996; Romana et al., 1996; Eguchi-Ishimae et al., 1998). Trisomy 21, as a secondary event, has been reported at frequencies of 14.6% (Loncarevic et al., 1999) and 17% (Raynaud et al., 1999).

We analyzed the 14 cases described here for secondary changes by interphase FISH (unpublished data). The observed abnormalities included duplication of the fusion gene (n = 3), deletion of the non-rearranged *TEL* allele (n = 9), trisomy 21 (n = 2), and monosomy X (n = 3), in agreement with previous reports (Loncarevic et al., 1999; Raynaud et al., 1999). With the exception of the fusion gene duplication, in which all three cases positively backtracked, other observable secondary features were noted in cases both positive and negative for *TEL-AML1* fusion. Interphase FISH also provided additional information about subclonal populations within diagnostic patient bone marrow samples. In the majority of informative cases (three had a single clone with several changes for which a sequence of events could not be discerned), t(12;21) appeared to be the initiating event, followed by the secondary events described above.

In conclusion, our findings confirm the in utero origin of at least 50% of cALL cases with *TEL-AML1*. We report a faster, more streamlined approach to characterizing the genomic breakpoints. We also confirm previous reports of the likely sequence of secondary events in the development of overt leukemia.

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