14q32 Translocations Are Associated With Mixed-Lineage Expression in Childhood Acute Leukemia

By Yasuhide Hayashi, Ching-Hon Pui, Frederick G. Behm, Ann H. Fuchs, Susana C. Raimondi, Geoffrey R. Kitchingman, Joseph Mirro, Jr, and Dorothy L. Williams

The frequency and characteristics of childhood acute leukemia with a 14q32 translocation [other than the t(8;14)(q24;q32)] were determined in 335 cases of newly diagnosed acute lymphoblastic leukemia (ALL) and 105 cases of acute nonlymphoblastic leukemia (ANLL). Ten children, representing 2.3% of the entire cohort, had this abnormality. (1.5% of ALL patients and 4.8% of ANLL patients). By French-American-British (FAB) criteria, 4 cases were classified as L1, 1 as L2, 2 as M1, 1 as M2, and 2 as M5. Remarkably, mixed-lineage expression was found in 6 of these 10 cases, but in only 21 of the other 430 cases without a 14q32 translocation (P < .001). Leukemic cells from 5 of these 6 cases (4 ANLL and 1 ALL) coexpressed CD13, a myeloid-associated antigen, and CD2. A T-cell-associated antigen (IgH) is located in this region. However, 14q32 abnormalities have also been reported in adults with T-cell lymphoma/leukemia, T-cell lymphoma, T-cell chronic lymphocytic leukemia (CLL), mycosis fungoides, and, rarely, acute nonlymphoblastic leukemia (ANLL).

We report here 10 children with acute leukemia and 14q32 translocations other than t(8;14)(q24;q32), which has been well-studied in Burkitt's lymphoma/leukemia. Remarkably, six of these patients had mixed-lineage characteristics. These results extend the finding of 14q32 translocations to acute mixed-lineage leukemia and suggest that an unidentified important gene may reside in this chromosomal region.

MATERIALS AND METHODS

From June 1983 to June 1988, we successfully analyzed leukemic cell karyotype and immunophenotype from 440 children with newly diagnosed acute leukemia, including 105 with ANLL and 335 with ALL. In this group, 14q32 translocations other than t(8;14)(q24;q32) were observed in five patients with ANLL (4.8%) and five with ALL (1.5%). These 10 patients are the subjects of this report. The clinical data of patients 1, 2, and 3 have been reported previously. Informed consent was obtained from the patients or their parents, and these investigations were approved by the institution's Clinical Trials Review Committee.

Morphologic and cytochemical studies. Bone marrow smears were stained with Wright-Giemsa, periodic acid-Schiff (PAS), myeloperoxidase (MPO), Sudan black B (SBB), and alpha-naphthyl butyrate esterase (ANB). Morphologic classification followed French-American-British (FAB) criteria.

Chromosome analysis. Bone marrow samples were processed according to the method of Williams et al; additional 24-hour cultures were also performed when samples were available. G-banding was done by treatment with trypsin and staining with Wright stain. Chromosome abnormalities were classified according to the International System for Human Cytogenetic Nomenclature (1985).

Immunophenotyping. Cell-surface antigens were detected by a standard indirect immunofluorescence assay with monoclonal antibodies to lymphoid-associated antigens, including J5 (common ALL antigen [CALLA], CD10), T11 (CD2), T1 (CD5), T3 (CD3), Leu-9 (CD7), B1 (CD20), and B4 (CD19), as well as myeloid-associated antigens, including MY1 (CD15), MY4 (CD14), MY7 (CD13), MY9 (CD33), and SF1 (CD36). After being stained, the leukemic cells were analyzed by fluorescence microscopy or with an
EPICS C flow cytometer equipped with a 5-W coherent laser (Coulter, Hialeah, FL). Staining of greater than 25% of the cells was considered a positive result. To demonstrate simultaneous expression of lymphoid- and myeloid-associated antigens by individual blasts, we examined cells from patients 3 and 10 with two-color immunofluorescence, using the EPICS C flow cytometer and the fluorescence isothiocyanate (FITC)-phycoerythrin (PE) dual-immunofluorescence protocol. Normal human lymphocytes dual-labeled with Leu 3a (CD4) conjugated to FITC and Leu 2c (CD8) conjugated to PE (Simultest, Becton Dickinson, Mountain View, CA) were used to correct for fluorescence spectrum overlap. In all experiments, negative controls included irrelevant mouse Ig directly conjugated to FITC or PE. CD2 antibody conjugated to FITC (T11-FITC) and CD13 antibody conjugated to PE (MY7-PE) (Coulter Immunology) were from commercial sources. Blast cells were also tested for surface Ig (sIg) and cytoplasmic Ig (cIg), which was considered positive if greater than 10% of cells were positive. Terminal deoxynucleotidyl transferase (TdT), detected by an immunofluorescence assay, was considered positive if more than 15% of cells demonstrated nuclear fluorescence.

**Classification of acute mixed-lineage leukemia.** The classification of ANLL and ALL followed FAB criteria. Cases of ANLL were considered to have mixed-lineage expression if the leukemic cells coexpressed two or more B-lineage-associated antigens (eg, CD19, CD20, CD22, sIg, cIg), or two or more T-lineage-associated antigens (eg, CD2, CD3, CD5, CD7). Similarly, the mixed-lineage expression in ALL cases was based on the coexpression of two or more myeloid-associated antigens (eg, CD11b, CD13, CD14, CD33, CD36).

**Southern blot analysis.** High molecular weight (mol wt) DNA was probed as described previously. BamHI, EcoRI, and HindIII digestions were used to analyze IgH genes; the probe consisted of a 3.4-kilobase (kb) EcoRI/HindIII fragment of the joining region (JH) that detects an 18-kb BamHI, a 17-kb EcoRI, and a 12-kb HindIII germ line fragment. BamHI digestions were also probed with a 2.5-kb γ light chain gene fragment that recognizes a 1.7-kb germ line fragment. TCR γ gene rearrangements were analyzed after BamHI, EcoRI, or XbaI digestion of high mol wt DNA by probing with pB400, a 0.4-kb cDNA containing sequences for Cγ3.

**RESULTS**

The pertinent clinical and laboratory features of the 10 cases with 14q32 translocation are shown in Table 1. The five boys and five girls ranged in age from 2 to 18 years (median, 5.9 years). By FAB criteria, cases 1 to 5 were classified as ANLL (M1 in 2 cases, M2 in 1, and M5 in 2) and cases 6 to 10 as ALL (L1 in 4 cases and L2 in 1). Although the three cases with M1 or M2 leukemia had positive MPO, the reactivity was low (less than 15%) in two of them. Initial central nervous system leukemia was present in patient 5 and a mediastinal mass in patient 10. Patients 1 through 5 received myeloid-directed induction treatment; two (nos. 3 and 4) failed to respond to the initial therapy. Patient 3 subsequently achieved a complete remission with lymphoid-directed induction therapy. Prolonged remissions have been maintained in patient 1 (30+ months), who underwent syngeneic bone marrow transplant in first remission, and in patient 3 (45+ months). All five patients with FAB-defined ALL attained a complete remission after lymphoid-directed induction therapy and remain disease-free for 1+ to 56+ months.

**DISCUSSION**

Although several cases with features of both the T-cell and myeloid lineages have been reported, no consistent chromosome abnormalities have been identified. This study suggests that translocations involving 14q32 may be one of the nonrandom chromosomal abnormalities in acute mixed-
Fig 1. G-banded partial karyotypes of cells from patients with balanced reciprocal translocations. The normal chromosomes are shown on the left and the rearranged ones on the right of each pair. Arrows indicate breakpoint sites. (A) Patient 7; (B) patient 3; (C) patient 2; (D) patient 6; (E) patient 9; (F) patient 10.
14q32 TRANSLOCATIONS IN ACUTE LEUKEMIA

Table 1. Clinical and Laboratory Features of the 10 Cases With 14q32 Translocations

<table>
<thead>
<tr>
<th>Feature</th>
<th>FAB-Defined ANLL Cases</th>
<th>FAB-Defined ALL Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAB</td>
<td>M5</td>
<td>M2</td>
</tr>
<tr>
<td>Age (y)</td>
<td>1.8</td>
<td>14.4</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>14.4</td>
<td>12.4</td>
</tr>
<tr>
<td>Leukocyte count (x 10^9/L)</td>
<td>18.2</td>
<td>6</td>
</tr>
<tr>
<td>Platelet count (x 10^9/L)</td>
<td>9.2</td>
<td>7.6</td>
</tr>
<tr>
<td>Liver (cm)†</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Spleen (cm)†</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Bone marrow blasts (%)</td>
<td>30</td>
<td>45%</td>
</tr>
<tr>
<td>MPO</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>SBB</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ANB</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Remission duration (mos)</td>
<td>30+‡</td>
<td>8</td>
</tr>
</tbody>
</table>

*Presence of Auer rods.
†Edge palpable below the costal margin.
‡Underwent syngeneic bone marrow transplant in first remission.
### Table 3. Chromosomal Findings in 10 Cases of Acute Leukemia With 14q32 Translocation

<table>
<thead>
<tr>
<th>Patient</th>
<th>No. of Metaphase Cells Examined</th>
<th>Karyotype</th>
<th>% of Normal Karyotype</th>
<th>Karyotype [% of abnormal metaphases]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>47,XY, +21, -14, +der(14)(t1;14)(q23;q32),i(7q)</td>
<td>100</td>
<td>1</td>
<td>47,XY, +21, +der(14)(t1;14)(q23;q32)</td>
</tr>
<tr>
<td>2</td>
<td>46,XX,t(14)(q12;q32)</td>
<td>94</td>
<td>9</td>
<td>46,XX,t(14)(q12;q32)</td>
</tr>
<tr>
<td>3</td>
<td>46,XX,t(16)(q23;q25;q32)</td>
<td>91</td>
<td>9</td>
<td>46,XX,t(16)(q23;q25;q32)</td>
</tr>
<tr>
<td>4</td>
<td>46,XY,-14,-der(14)(t1;14)(q32;q32)</td>
<td>99</td>
<td>9</td>
<td>46,XY,-14,-der(14)(t1;14)(q32;q32)</td>
</tr>
<tr>
<td>5</td>
<td>46,Y,-X,+mar,-11,+der(11)(t11;?)(q13;?),-14,+der(14)(t14;7)(q32;?)</td>
<td>100</td>
<td>1</td>
<td>46,Y,-X,+mar,-11,+der(11)(t11;?)(q13;?),-14,+der(14)(t14;7)(q32;?)</td>
</tr>
<tr>
<td>6</td>
<td>46,XX,t(14;4;7)(q21;q32;p15)</td>
<td>44</td>
<td>4</td>
<td>46,XX,t(14;4;7)(q21;q32;p15)</td>
</tr>
<tr>
<td>7</td>
<td>45,XX,-12,-15,+dic(12;15)(p11;?),t(12;14)(q24;q31;q32)</td>
<td>18</td>
<td>1</td>
<td>45,XX,-12,-15,+dic(12;15)(p11;?),t(12;14)(q24;q31;q32)</td>
</tr>
<tr>
<td>8</td>
<td>45,XY,-8,-14,+der(14)(t8;14)(q23;?),del(5)(p14),del(17)(p12)</td>
<td>33</td>
<td>3</td>
<td>45,XY,-8,-14,+der(14)(t8;14)(q23;?),del(5)(p14),del(17)(p12)</td>
</tr>
<tr>
<td>9</td>
<td>46,XY,-15,-der(15)(t15;?),t(8;14)(q11;q32),t(9;22)</td>
<td>10</td>
<td>1</td>
<td>46,XY,-15,-der(15)(t15;?),t(8;14)(q11;q32),t(9;22)</td>
</tr>
<tr>
<td>10</td>
<td>47,XY,+t(12;14)(q13;q32)</td>
<td>67</td>
<td>6</td>
<td>47,XY,+t(12;14)(q13;q32)</td>
</tr>
</tbody>
</table>

Although 14q+ or t(5;14)(q32;q32) has been reported in ALL patients presenting with hypereosinophilia,33,54 neither abnormality was identified in this study.

The reason for the mixed-lineage expression among cases with 14q32 translocation is unknown. The lack of rearrangement of the IgH gene in our mixed-lineage cases suggests that the breakpoints of 14q32 did not involve the JH, region of the IgH gene. Nevertheless, it is still possible that the enhancer or promotor of the IgH gene may be involved in the development of these cases. Another possibility is the involvement of a putative oncogene35-37 or the proto-oncogene (AKT-118 or ELK-225) located in the 14q32 region. Some of these genes have been localized to a region centromeric to the IgH gene region,55,56,58 which is located at 14q32.3. Finally, a myeloid or T-lymphoid-associated gene, or both may reside in the 14q32 region. Further molecular studies are needed to clarify this issue.

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**REFERENCES**


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