INVolvement of the **MLL/ALL-1** GENE ASSOCIATED WITH MULTIPLE POINT MUTATIONS OF THE N-ras GENE IN ACUTE MYELOID LEUKEMIA WITH t(11;17)(q23;q25)

To the Editor:

Chromosome 11 band q23 (11q23) is one of the most frequent disrupting regions of the chromosome translocations and deletions in various types of leukemia, lymphomas, and myelodysplastic syndromes. The partner regions for the reciprocal translocations are 1p32, 1q21, 2p21, 4q21, 6q27, 9p22, 10p15, 14q32, 17q25, and 19p13. Recent studies have shown that the **MLL/ALL-1** gene is involved in the t(4;11)(q21;q23) and t(11;19)(q23;p13) translocations of infantile acute leukemia with biphenotypic character. Furthermore, this gene also associated with t(1;11), t(6;11) and t(9;11). The **MLL/ALL-1** gene encodes for a human homologue of the Drosophila trithorax protein that fuses to the partner chromosome-encoded protein, producing a fusion transcriptional factor. Thus, the **MLL/ALL-1** gene is likely to be associated with leukemogenesis in the 11q23 translocation. We present here a case of acute myeloid leukemia (AML) with the t(11;17)(q23;q25) translocation showing the rearrangement of the **MLL/ALL-1** gene associated with multiple N-ras gene mutations.

A 9-year-old girl was referred to us for AML (M1) according to the French-American-British (FAB) classification, with leukocyte count of $85 \times 10^3/\mu L$, hemoglobin (Hb) 8.1 g/dL, and platelets $30 \times 10^3/\mu L$. She had been healthy except some episodes of viral infection. She received combination chemotherapy and achieved complete remission. However, 1.5 years later, the AML relapsed despite the intermittent intensification, and she died of cerebral bleeding. Karyotype analysis of the blast cells at diagnosis showed 46 XX with the t(11;17)(q23;q25) translocation in 20 of 20 analyzed cells. The breakpoints of the **MLL/ALL-1** gene have been clustered within the BamHI 9-kb genomic fragment, which can be detected by the BamHI 0.9-kb cDNA fragment (Fig 1). Southern blot analysis using this probe showed two rearranged bands in the BamHI digested DNA, and one rearranged band accompanied with a relatively fainter germ line band at 5.5 kb in the EcoRV digested DNA (Fig 1), which suggested that the **MLL/ALL-1** gene was disrupted within the EcoRV-BamHI 5-kb fragment and jointed presumably with a DNA fragment on 17q25. Furthermore, the blast cells at diagnosis had the multiple-point mutations of the N-ras gene. cDNA of the N-ras gene were amplified by reverse transcriptase-polymerase chain reaction (RT-PCR) and were cloned into the MI3mpl8 vector. The presence of mutations in 90 clones was analyzed by means of oligonucleotide hybridization (Table 1). Two clones containing double-loci mutations at codons 13 and 61 were detected in the same allele. Forty clones contained each mutation at codons 12, 13, and 61, and the remaining 48 clones showed the wild-type of N-ras gene. Southern blot analysis of the N-ras gene

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**Fig 1.** Southern blot analysis with a probe of the partial **MLL/ALL-1** cDNA. The illustrated cDNA probe detects 9-kb and 9/5.5-kb fragments of control DNA (C) digested by BamHI (B) and EcoRV (V), respectively. The rearranged bands in the patient DNA (P) are indicated by arrows.
Table 1. Clonal Analysis for the Presence of N-ras Point Mutations With Oligonucleotide Hybridization and Sequencing

<table>
<thead>
<tr>
<th>Point Mutation</th>
<th>No. of Clone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type (12:GGT, 13:GGT, 61:CAA)</td>
<td>48</td>
</tr>
<tr>
<td>Codon 12:GAT</td>
<td>3</td>
</tr>
<tr>
<td>Codon 13:CGT</td>
<td>7</td>
</tr>
<tr>
<td>Codon 13:GAT</td>
<td>26</td>
</tr>
<tr>
<td>Codon 61:CAc</td>
<td>4</td>
</tr>
<tr>
<td>Codons 13:CGT and 61:CAc</td>
<td>1</td>
</tr>
<tr>
<td>Codons 13:GAT and 61:CAc</td>
<td>1</td>
</tr>
</tbody>
</table>

* Underlines indicate the substituted nucleotides.

Data from Kubo et al. showed neither rearrangement nor change of the copy number (data not shown).

This case showed that the MLL/ALL-1 gene is a target gene for the t(11;17)(q23;q25) translocation. In regard to a fused transcript between the MLL/ALL-1 gene and the novel gene on 17q25, transcripts could not be analyzed because of the small amount of the sample. Additionally, multiple clonality of the N-ras gene was demonstrated in contrast to the monoclonal MLL/ALL-1 gene rearrangement. These findings suggest that the multiple N-ras mutations might occur posterior to the chromosomal translocation but not be characterized simply by an accumulative process.

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REFERENCES

Involvement of the MLL/ALL-1 gene associated with multiple point mutations of the N-ras gene in acute myeloid leukemia with t(11;17)(q23;q25) [letter]