Frequent Loss of Heterozygosity on the Long Arm of Chromosome 6: Identification of Two Distinct Regions of Deletion in Childhood Acute Lymphoblastic Leukemia

Seisuo Takeuchi, Michiaki Kolke, Taku Seriu, Claus R. Bartram, Martin Schrappe, Alfred Reiter, Susan Park, Harry E. Taub, Ichiro Kubonishi, Isao Miyoshi, and H. Phillip Koeffler

ABSTRACT

Cytogenetic analysis of childhood acute lymphoblastic leukemia (ALL) identified nonrandom chromosomal abnormalities of the long arm of chromosome 6. Most of the alterations are deletions that are thought to be indicative of the presence of a tumor suppressor gene that is mutated on the remaining allele. These observations led us to consider whether 6q loss may contribute to the pathogenesis of childhood ALL. To define further a region containing this gene, we analyzed the loss of heterozygosity (LOH) of chromosome 6 in 113 primary ALL samples with matched normal DNA using 34 highly informative microsatellite markers. LOH was found in 17 (15%) samples at one or more of the loci, and partial or interstitial deletions of 6q were detected in 11 of these tumors. On the basis of these results, we performed a detailed deletional map and identified two distinct regions of deletion. The first region is flanked by D6S283 and D6S302 loci at 6q21-22. The second region is flanked by D6S275 and D6S283 loci at 6q21. Clinical analysis determined that LOH of 6q was demonstrated both in precursor-B cell ALLs (15 of 93; 16%) and in T cell ALLs (2 of 19; 11%). In addition, 19 patients have been studied at diagnosis and relapse; 18 showed the same 6q21-22 structural abnormality at relapse (normal, 16 patients; LOH, 2 patients) as their initial presentation, suggesting, albeit with a small patient sample size, that 6q21-22 deletions may be an initial event in leukemogenesis and may occur less frequently during the progression of childhood ALL. These data suggest the presence of putative tumor suppressor genes on chromosome arm 6q that are important in the development of both T and precursor-B childhood ALLs. Our map provides important information toward cloning putative ALL tumor suppressor genes.

INTRODUCTION

Cytogenetic studies have been frequently performed in childhood ALL and have identified nonrandom translocations and deletions (1–6). Like other tumors, ALL probably results from accumulation of genetic alterations that affect normal control of cellular growth. Malignant transformation can arise as a result of the increased activity of growth-promoting genes (oncogenes) and/or the inactivation or loss of growth-constraining genes (tumor suppressor genes). Inactivation or deletion of tumor suppressor genes is a major mechanism of tumorigenesis in a variety of tissues. Several molecular alterations have been described in childhood ALL. LOH of the short arm of chromosome 9 was found in 40% of patients, and deletions of the CDKN2/INK4A/p16 gene occur in about 15% of precursor-B-ALLs and 75% of T-ALLs (7–14). LOH of the short arm of chromosome 12 was found in 17–33% and TEL-AML1 fusion in 16–27% of childhood B-lineage ALL patients (7, 15–22).

Abnormalities of the long arm of chromosome 6 are commonly observed in childhood ALL. Most of the abnormalities are deletions (3, 23). Cytogenetic deletions of the long arm of chromosome 6 were reported in 4–13% of patients with childhood ALL (3, 23). These observations are thought to be indicative of the presence of a tumor suppressor gene on 6q that is mutated on the remaining allele. However, the frequency and region of the deletions is difficult to estimate by conventional cytogenetic analysis because small interstitial deletions are beyond the sensitivity of the technique. Molecular analysis of the long arm of chromosome 6 is limited in scope. The paradigm of alterations of several tumor suppressor genes is a mutation of one allele and loss of the second allele. This reduction to homozygosity in the region of the tumor suppressor gene can be detected as a LOH of informative markers in the region of the tumor suppressor gene. Thus, LOH analysis is an indirect method to search for a tumor suppressor gene.

In the present study, we have analyzed LOH of chromosome arm 6q in 113 primary ALL samples with matched normal DNA using 34 highly polymorphic microsatellite markers. We identified two distinct regions of deletion on the long arm of chromosome 6.

MATERIALS AND METHODS

Samples. One hundred thirteen primary ALL DNAs were obtained from patients of the ongoing Multicenter Trial ALL-Berlin-Frankfurt-Münster 90 of childhood ALL of the German Berlin-Frankfurt-Münster group (diagnosis between April 1990 and April 1994). Informed consent was obtained from the patients, their parents, or both, as appropriate. The technique of immunophenotyping has been described previously (24). The percentage of blast cells was at least 80% and usually more than 90%. The corresponding normal DNAs were obtained from the bone marrow blood after CR was achieved (at least 12 months after initial diagnosis). All of the CR samples were examined for minimal residual disease by PCR-based assay. All of the CR samples used in this study were PCR negative. Sensitivity of this assay is between 10^{-4} and 10^{-6}. The remaining DNA samples were obtained from 11 patients at the time of their relapse. Cytogenetic data were only available from nine patients. Five cases revealed a normal karyotype (cases 12, 38, 45, 49, and 63), and four cases showed chromosomal abnormalities: case 8: 46,XY,t(9;22); case 16: 52,XY,+X,+4,+5,+6,+10,+18,+21; case 51: 47,XY,+21(t;8;12)(p11;p12); and case 67: 46,XY,del(6)(q21).

Analysis of LOH Using Microsatellite Markers. The LOH analysis was performed by PCR-amplification of microsatellite sequences. The genetic map of chromosome 6 was compiled from the Généthon microsatellite map (25, 26). Some markers have been assigned to the same location in a 0-cM cluster. Primers were obtained from Research Genetics (Huntsville, AL).

Each PCR contained 5–25 ng of DNA, 10 pmol of each of the primers, 2 pmol of each of the four deoxyribonucleotide triphosphates (Amersham Pharmacia Biotech, Stockholm, Sweden), 0.5 units of Taq DNA polymerase (Boehringer Mannheim, Indianapolis, IN), and 2 μCi of [α-32P]dCTP (ICN, Irvine, CA) in 20 μl of the specified buffer with 1.5 mM MgCl₂. Samples were amplified by 30–35 cycles of denaturing for 40 s at 94°C, annealing for 30 s at 55°C, and extending for 1 min at 72°C in a Programmable Thermal Controller (M. J. Research, Inc., Watertown, MA). After amplification, PCR

Received 10/21/97; accepted 4/10/98.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Supported in part by NIH grants, the Concern Foundation, the Parker Hughes Trust (to H. P. K.), the Deutsche Forschungsgemeinschaft, Deutsche Krebshilfe (to C. R. B.), and a grant-in-aid from the Ministry of Education, Science, Sports, and Culture of Japan (to S. T.).

2 To whom requests for reprints should be addressed, at Department of Internal Medicine, Kochi Medical School, Okochobo, Nankoku, Kochi 783, Japan. Phone: 81-888-80-2345; Fax: 81-888-80-2348.

3 The abbreviations used are: LOH, loss of heterozygosity; ALL, acute lymphoblastic leukemia; B-ALL, B cell ALL; T-ALL, T cell ALL; CR, complete remission; NHL, non-Hodgkin’s lymphoma.
samples were diluted 5-fold in loading buffer containing 20 mM EDTA, 96% formamide, and 0.05% of both bromophenol blue and xylene cyanol. The products were heated to 95°C for 5 min, and 1.5 μl of each sample were electrophoresed through a 6% polyacrylamide gel containing 8.3 M urea for 3-4 h at 85 W. Subsequently, the gels were dried and subjected to autoradiography using Kodak XAR film at -80°C. LOH was inferred only when substantial reduction was measured in the ratio of allele radiographic signal intensities in the tumor sample relative to that in the corresponding normal sample.

RESULTS

Analysis of LOH Using Microsatellite Markers. We first screened 48 samples for LOH of chromosome 6 using 25 microsatellite markers. Figs. 1 and 2 display representative autoradiographs showing LOH. Six samples showed LOH at least at one locus on chromosome 6. The pattern of LOH in each patient is shown in Fig. 3. Of these 6 cases, three of them (cases 5, 29, and 30) showed LOH at all informative loci on chromosome 6 (Fig. 3). This is consistent with loss of whole chromosome 6 in these three cases. One very informative sample (case 12) lost alleles at D6S275/D6S417 but maintained heterozygosity both at the distal neighboring locus.

Fig. 1. Representative autoradiographs showing LOH in the region of D6S268 and D6S278. Sample 23 shows LOH at D6S268, with apparent retention of heterozygosity at D6S278. Sample 24 shows LOH at D6S302, with apparent retention of heterozygosity at D6S283. Sample 65 shows LOH at D6S268, D6S278, and D6S302. T, tumor DNA; N, normal DNA.

Fig. 2. Representative autoradiographs showing LOH in the region of D6S300/D6S424 and D6S468. Sample 55 shows LOH at D6S300, with apparent retention of heterozygosity at D6S283. Sample 127 shows LOH at D6S468, with apparent retention of heterozygosity at D6S283. Sample 129 shows LOH at D6S275 and D6S300, with apparent retention of heterozygosity at D6S283. T, tumor DNA; N, normal DNA.

D6S268, D6S283, D6S300, and D6S302 show evidence of deletion. The other two samples with relatively large interstitial deletions (cases 23 and 36) also had LOH at D6S275/D6S417. Taken together, a commonly deleted region on chromosome arm 6q was determined between D6S284/D6S286 and D6S287/D6S304. The other two samples with relatively large interstitial deletions (cases 23 and 36) also had LOH at D6S275/D6S417. Taken together, a commonly deleted region on chromosome arm 6q was determined between D6S284/D6S286 and D6S267/D6S303/D6S304.

On the basis of these results, we next performed a detailed deletional map and identified two distinct regions of deletion on 6q. We used 19 highly informative microsatellite markers between D6S284/D6S286 and D6S262 and examined 113 ALL samples, including the initial 48 samples described above. The pattern of LOH for each positive patient is shown in Fig. 4. From the first set of 48 samples used for our initial screening, we identified an additional sample with LOH (case 24). This sample had so small a deletion that it was missed by the initial screening. In total, 17 samples showed LOH at least at one locus on chromosome arm 6q. Of these 17 cases, 6 (cases 5, 29, 30, 118, 119, and 143) showed LOH at all informative loci on chromosome arm 6q. The other 11 cases showed interstitial deletions of chromosome arm 6q. Four samples (cases 12, 23, 36, and 67) had relatively large interstitial deletions. Seven samples (cases 24, 55, 65, 68, 115, 127, and 129) had small deletions. All of the samples that displayed LOH had a single region of deletion.
Fig. 3. Summary of LOH screening on chromosome 6 in childhood ALL. Forty-eight samples were screened for LOH of chromosome 6. Six samples that showed LOH at one or more loci are presented. The genetic map of chromosome 6 was compiled from the Généthon microsatellite map. For the markers that are assigned to the same location by linkage analysis, the results obtained from each marker were combined. Status of each chromosome locus is indicated by shading as LOH (◻), retention of heterozygosity (■), and not informative (■). Patient numbers are listed at the top of each column.

Four very informative samples (cases 23, 24, 65, and 68) had LOH in the region of D6S268 and D6S278. Sample 23 maintained heterozygosity at D6S302 but had LOH at the proximal locus D6S268. Sample 24 maintained heterozygosity at D6S263/D6S449 but had LOH at the distal locus D6S302 (Figs. 1 and 4). Therefore, the smallest commonly deleted region is between D6S263/D6S449 and D6S302. All four of the samples (cases 23, 24, 65, and 68) had LOH in the region. The other three samples (cases 12, 36, and 67) with relatively large interstitial deletions also had LOH in this region. Thirteen of 17 samples with deletions lost this region. The genetic distance between these two markers is 9 cM. Taken together, a tumor suppressor gene probably exists within the smallest commonly deleted region, flanked by D6S263/D6S449 and D6S302; this region has a size of 9 cM.

The other very informative samples (cases 55, 115, 127, and 129) had LOH in the region of D6S300/D6S424 and D6S468. Sample 55 maintained heterozygosity at D6S267/D6S417 but had LOH at the distal loci D6S300/D6S424. Sample 127 maintained heterozygosity at D6S268 but had LOH at the proximal locus D6S468 (Figs. 2 and 4). Therefore, the smallest commonly deleted region is between D6S267/D6S417 and D6S268/D6S449. All four of the samples (cases 55, 115, 127, and 129) had LOH in the region. The other three samples (cases 12, 36, and 67) with relatively large interstitial deletions also had LOH in this region. Fourteen of 17 samples with deletions lost this region. The genetic distance between these two markers is 8 cM. Taken together, a tumor suppressor gene probably exists within the smallest commonly deleted region, flanked by D6S267/D6S417 and D6S268/D6S449, which is separated by a distance of 8 cM.

Analysis of LOH of 6q at the Time of Relapse. DNA samples were obtained at the time of relapse from 19 patients (cases 2, 3, 5, 6, 15, 18, 23, 37, 50, 53, 62, 64, 65, 66, 72, 83, 87, 118, and 131) and were analyzed for LOH of 6q at 22. The following microsatellite loci were used: D6S284, D6S246, D6S275, D6S417, D6S300, D6S424, D6S468, D6S268, D6S283, D6S449, D6S268, D6S278, D6S302, D6S266, and

Fig. 4. Summary of detailed LOH map on chromosome 6 in childhood ALL. Detailed LOH map of chromosome 6 was performed between D6S284 and D6S262 using 113 samples. Seventeen samples that showed LOH at one or more loci are presented. The genetic map of chromosome 6 was compiled from the Généthon microsatellite map. For the markers that are assigned to the same location by linkage analysis, the results obtained from each marker were combined. Status of each chromosome locus is indicated by shading as LOH (◻), retention of heterozygosity (■), and not informative (■). Patient numbers are listed at the top of each column.
LOH of 6q in Childhood ALL

Table 1 Clinical characteristics of 112 ALL patients with and without 6q LOH

<table>
<thead>
<tr>
<th>Clinical features at diagnosis</th>
<th>No. of patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total (%) (n = 112)</td>
</tr>
<tr>
<td>Immunophenotype</td>
<td></td>
</tr>
<tr>
<td>T-ALL</td>
<td>19 (17)</td>
</tr>
<tr>
<td>Precursor-B-ALL</td>
<td>93 (83)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>64 (57)</td>
</tr>
<tr>
<td>Female</td>
<td>48 (43)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
</tr>
<tr>
<td>&lt;1</td>
<td>3 (3)</td>
</tr>
<tr>
<td>1–10</td>
<td>91 (81)</td>
</tr>
<tr>
<td>&gt;10</td>
<td>18 (16)</td>
</tr>
<tr>
<td>WBC count (10^9/l)</td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>55 (49)</td>
</tr>
<tr>
<td>&gt;20</td>
<td>57 (51)</td>
</tr>
<tr>
<td>Response</td>
<td></td>
</tr>
<tr>
<td>Poor</td>
<td>15 (13)</td>
</tr>
<tr>
<td>Good</td>
<td>94 (84)</td>
</tr>
<tr>
<td>Relapse</td>
<td>30 (28)</td>
</tr>
</tbody>
</table>

Note: Precursor-B-ALL includes pre-B and common ALL.

D65303. The status of 6q loss at initial presentation is as follows: three patients (cases 5, 23, and 65) had LOH of 6q. The other 16 patients had a normal chromosome 6. Two patients (cases 5 and 65) showed LOH at the time of relapse as well as at their first presentation. LOH of sample from patient 23 was not detectable at the time of relapse. All 16 of the patients with normal 6q at their first presentation also had a normal 6q at the time of their relapse. In total, 18 of 19 patients showed the same 6q21-22 structure at relapse as their initial presentation, suggesting that 6q deletion may be an initial event in leukemogenesis and may occur less frequently during the progression of childhood ALL.

Clinical Characteristics of the Patients with 6q LOH. Clinical information was available for 112 patients examined in this study (Table 1). Nineteen patients had T-ALL, and 93 patients had precursor-B-ALL. LOH of chromosome arm 6q was found in both precursor-B-ALLs (15 of 93; 16%) and T-ALLs (2 of 19; 11%). No statistically significant associations were found between 6q LOH and sex. Six percent of the patients with 6q LOH were over age 10 at the time of diagnosis, compared to 18% in patients with normal 6q. Concerning WBC counts at diagnosis, it appears that patients with 6q LOH have a lower WBC count at diagnosis: 71% with WBC count < 20,000/μl, compared with 45% in patients with normal 6q. However, due to the small number of patients, there are no statistically significant differences. All 112 of the patients were treated uniformly, and 6% of the patients with 6q LOH showed the poor response to initial induction chemotherapy, compared to 15% patients with normal 6q. Of these 112 patients, 109 were eligible for follow-up. Patients with 6q LOH had the same incidence of relapse (31%) as the cohort with normal 6q (27%) at 5 years after initiation of therapy (Fig. 5).

DISCUSSION

To understand the role of genetic changes in the development of ALL, we performed detailed deletion mapping of chromosome 6 using highly informative markers. We have found LOH on 6q in 15% of ALLs. This is higher than the frequency of cytogenetic deletions of 6q (4–13%) in ALL (3, 23). Thus, cytogenetic studies have probably missed some cases of small interstitial deletions on 6q. We previously examined LOH of 9p and 12p using the same series of childhood ALL samples as used in this study, and we found that LOH of these two arms was also much more frequent than the reported frequency of cytogenetic deletions (7, 11, 13, 18). These results show the power of LOH analysis using microsatellite markers.

LOH analysis of 6q in childhood ALL has been limited in scope. We previously performed allelotype analysis of childhood ALL and found LOH of 6q in 3 of 24 (13%) patients (7). Cavel et al. (27) found LOH of 6q in one of 42 (2%) patients in their allelotype analysis. However, the number of microsatellite markers that were used and the number of patients that were examined were too small in both studies to identify the critical chromosomal region of deletion.

Previous cytogenetic analysis of childhood ALL identified the common region of deletion as the band at 6q21-22 (23). We found two distinct regions of deletion on 6q. The first was between D6S283 and D6S302; it has a size of 9 cM. This region was previously identified by Gérard et al. (28) using the same technique as ours. Recently, this region was further narrowed to a 2-megabase segment by fluorescence in situ hybridization analysis (29, 30). The present study confirmed those findings. Moreover, we identified a second region of deletion, which has not been documented previously. It is between D6S275 and D6S283 and has a size of 8 cM. Again, this shows the power of LOH analysis using microsatellite markers. This map provides important information toward cloning these putative ALL suppressor genes.

In hematomal malignancies, deletions involving the long arm of chromosome 6 are observed primarily in lymphoid malignancies, i.e., ALL, lymphoproliferative disorder, and NHL (2). In NHL, two re-
gions of deletions have been identified (31, 32). The first region is at 6q21, which is also deleted in ALL. Deletion of the same region is also observed in secondary childhood ALL samples (15 of 93; 16%) and in T-ALLs (2 of 19; 11%), which probably has an important role in progression of childhood ALL. This is in contrast with p53 mutations, which were found more frequently at relapse and relapse in 19 patients. Eighteen of these 19 patients showed the same 6q21-22 structure as their first presentation, suggesting, albeit with a small patient sample size, that 6q21–22 deletions may be an initial event in leukemogenesis and may occur less frequently during the progression of childhood ALL. This is in contrast with p53 mutations, which were found more frequently at relapse and which probably has an important role in progression of childhood ALL to a more aggressive phenotype (38–40). However, the sample size of relapsed patients is too small at this time for statistical analysis of the data. Longer follow-up periods will be required for relapse of more patients. This parameter will be followed.

Clinical information was available for 112 patients in this study. Molecular deletions of chromosome arm 6q were found both in precursor-B-ALLs (15 of 93; 16%) and in T-ALLs (2 of 19; 11%), suggesting that the tumor suppressor gene in this region is critical to normal B- and T-cell function, and its alteration may allow uncontrolled growth of early lymphoid progenitor cells. This is in contrast to the homozygous deletions of the p15/p16 genes at 9p21, which are more frequently altered in T-ALL samples (8, 11). The TEL/AML-1 fusion and associated 12p LOH are more frequently observed in precursor-B-ALL samples than T-ALLs (22). All 112 patients were treated uniformly. We did not find any significant association between LOH of 6q and the observed proportion of treatment failures at 5 years. This parameter will be given a longer follow-up.

ACKNOWLEDGMENTS

We are grateful to Jochen Harbott for providing the cytogenetic data and Wolf Dieter Ludwig for providing immunophenotypic data. We thank Yoshihiro Hatta, Naoki Mori, Taizo Tasaka, and Hiroya Asou for helpful and valuable discussions. We also thank Marge Goldberg for excellent secretarial help.

REFERENCES


Frequent Loss of Heterozygosity on the Long Arm of Chromosome 6: Identification of Two Distinct Regions of Deletion in Childhood Acute Lymphoblastic Leukemia


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/58/12/2618

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.