Molecular Rearrangements on Chromosome 11q23 Predominate in Infant Acute Lymphoblastic Leukemia and Are Associated With Specific Biologic Variables and Poor Outcome

By Chien-Shing Chen, Poul H.B. Sorensen, Peter H. Domer, Gregory H. Reaman, Stanley J. Korsmeyer, Nyla A. Heerema, G. Denman Hammond, and John H. Kersey

Acute lymphoblastic leukemia (ALL) in infants generally shows distinctive biologic features and has a poor prognosis. Cytogenetic studies indicate that many infant leukemias have chromosome 11q23 translocations. Because of these findings and the distinct clinical features of infant leukemia, we investigated 30 cases of infant ALL for molecular defects of 11q23. Fourteen cases had cytogenetic abnormalities of 11q23, and all of them showed 11q23 rearrangements at the molecular level. An additional seven cases also had 11q23 molecular rearrangements, including one with normal cytogenetic analysis. Molecular abnormalities of 11q23 were significantly correlated with adverse prognostic factors, including age under 6 months, hyperleukocytosis, CD10− phenotype, and early treatment failure. Molecular analysis identified a group of infants with germline 11q23 that had a very good treatment outcome with a projected event-free survival of 80% at median follow-up of 46 months compared to 15% in infants with rearranged 11q23 (P < .001). These findings suggest that a high proportion (70%) of infants with ALL have 11q23 rearrangements and that these rearrangements are not always detectable by cytogenetic analysis. The presence of germline 11q23 DNA may identify a subgroup of infant ALL patients with a good outcome using current therapy and a different etiology for their ALL.

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11q23 MOLECULAR REARRANGEMENT IN INFANT ALL

Cell-surface antigens were detected by a standard indirect immunochemical assay. The diagnosis of ALL was based on standard French-American-British (FAB) morphologic and cytochemical criteria. The leukemias of all 30 cases were either L1 or L2 according to the FAB classification. Cell-surface antigens were detected by a standard indirect immunochemical assay using flow cytometry. The immunophenotyping was performed in CCSG reference laboratories at Children's National Medical Center, Washington, DC; the Fred Hutchinson Cancer Research Center, Seattle, WA; or the University of Minnesota, Minneapolis. Surface antigen expression was studied and the most frequently used antibodies were CD9 (BA-2), CD10 (BA-3), CD19 (BA-4), CD24 (BA-1), CD34 (MY10), and HLA-DR (12). Cell-surface antigens were considered positive for the purpose of analysis if they were expressed on 20% or more of the blast cells. All cases were B-lineage in origin as defined by HLA-DR, and CD19, or CD24 expression was analyzed stringently for the purpose of this study; to include a case as cytogenetically unremarkable at chromosome 11q23, at least 20 metaphase cells were required for analysis.

Statistical analyses: Several statistical methods were used to evaluate the various parameters studied in these infant leukemias. Comparisons of the various clinical and laboratory features with discrete variables between cases with and without rearrangement on 11q23 were performed using Fisher's exact test. Significance of the difference in the leukocyte count at the time of diagnosis between the two groups of patients was examined by the Mann-Whitney test.

Time-to-failure, defined as the interval (duration) between first remission and failure (relapse or death caused by any cause), or current follow-up for patients not failing (censored cases) was analyzed by the Kaplan-Meier procedure. Early death or failure to enter remission was considered a failure at zero time. Differences in failure-free survival were compared by the log rank test. Risk of an event was estimated using the observed to expected ratios for the two groups (rearrangement vs germline). The software used was "Epilog Plus," designed by Epicenter Software Inc, Pasadena, CA.

Genomic library screening and restriction mapping. In a related study, a CD3y yeast artificial chromosome (YAC) clone was isolated from the Washington University human genomic YAC library. The CD3y YAC was subcloned into λ phage and a phage contig was constructed. A panel of somatic-cell hybrids made from the RS 4;11 cell line was used to identify clones that span the RS 4;11 breakpoint. Single-copy probes centromeric and telomeric to the breakpoint (98.40 and P/S4, respectively) were subsequently isolated and used in the present study. Also used was 4.2E, a subclone of the germline EcoRI fragment located adjacent and telomeric to the EcoRI fragment recognized by P/S4 (see below). The positions of these probes are shown in Fig 1. In our study, a germline restriction map of the 11q23 breakpoint region was generated by screening a lung fibroblast WI 31 genomic library (Stratagene, San Diego, CA) with the 98.40 probe and two independent overlapping clones of the 11q23 region were identified. Restriction mapping of these two clones was performed by probing Southern blots of single- and double-restriction enzyme digests as well as by probing UV-irradiated and subsequently restriction-digested blots with α-P32-labeled T3 and T7 primers. Individual HindIII, EcoRI, SalI, and BamHI restriction enzyme sites were mapped in this region. All EcoRI insert fragments from these two λ phage clones were subsequently cloned into the Bluescript vector (Stratagene) so that these EcoRI fragments could be used as probes in 11q23 breakpoint mapping experiments (including 4.2E). These studies showed a 22-kb DNA region that is identified by all three probes (98.40, P/S4, 4.2E) used in these studies. Furthermore, all of the 11q23 breakpoints or rearrangements in the infant ALL cases studied were mapped within this cloned DNA fragment.

DNA preparation, pulsed-field gel electrophoresis (PFGE), and Southern analyses: Preparation of DNA in agarose blocks and restriction enzyme digestion for PFGE were described. Germline control DNA was isolated from either normal individual PBL or human placental DNA. One-percent agarose gels were run in a contour-clamped homogeneous electric fields (CHEF) PFGE electrode array constructed as described by Chu et al. High molecular weight DNA was digested, fractionated by CHEF, and then transferred to a nylon membrane (Nytran; Schleicher and Schuell, Inc, Keene, NH) in 10× SSPE (NaCl, NaH2PO4, and EDTA). The electrohoresis and blotting conditions have been described previously.

DNA samples were also extracted and prepared for conventional Southern analysis. Germline-control DNA was isolated from either normal individual PBL or human placental DNA. Southern blot analyses were performed according to standard methods. Membranes were stripped and re-exposed to film for an appropriate time before the hybridization with a different probe. To preamplify repetitive sequences recognized by the 4.2E probe during Southern analysis, 400 μg/mL of total human placental DNA (Sigma Chemical Co, St Louis, MO) was included in the hybridization solution.

RESULTS

Chromosome 11q23 is rearranged in the majority of infant ALL cases. In a previous long-range mapping study using pulsed-field gel analysis, we showed breakpoint clustering in t(4;11) acute leukemia. In the present study, we investigated the possibility that further fine resolution of 11q23 breakpoint clustering was detectable using probes described in the Materials and Methods. Cases of ALL in infants were analyzed by conventional Southern analysis of HindIII, EcoRI, and, in some cases, BamHI digests as well as by pulsed-field gel analysis of BssHII digests, using P/S4, 98.40, and 4.2E probes. The 11q23 rearrangements of the leukemia cases were mapped relative to the germline restriction map of 11q23 based on the analysis of the above enzyme digests. The results, summarized in Table 2, indicate that the rearrangements could be mapped to the three adjacent EcoRI DNA fragments shown in Fig 1. Among the 30 cases of ALL in infants studied, 21 (70%) had detectable 11q23 rearrangements that could be mapped within a 15-kb DNA region. Most of the rearrangements (13/21) occurred in the EcoRI fragment that was detected by the P/S4 probe (EcoB). Three cases (5, 7, and 17) had their breakpoints mapped to the EcoRI fragment recognized by 98.40 (EcoA) and four (2, 6, 8, and 16) were mapped to 4.2E region (EcoC). In case 19, rearrangements were shown in both HindIII and BssHII digests with either P/S4 or 98.40 probes but not in EcoRI digests. Possibly, the size of the rearranged band after EcoRI digestion is not distinguishable from that of the germline band by Southern analysis. Therefore, the breakpoint location for case 19 has not been assigned based on EcoRI digests. In cases 11 and
Table 1. Clinical Features and Treatment Outcomes of 30 Infants With Acute Lymphoblastic Leukemia

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Molecular rearrangement of 11q23

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Germline 11q23

21, two equally intense rearranged bands were observed in EcoR digests using the P/S4 probe, suggesting that the breakpoints occurred within the DNA region recognized by the P/S4 probe (data not shown). The molecular rearrangements in all cases studied were observed with at least two different enzyme digests, indicating that the rearranged bands seen were unlikely caused by the results of either germline restriction fragment-length polymorphism (RFLP) or partial digestion.

11q23 rearrangements are detected in infant ALL lacking cytogenetically detectable 11q23 abnormalities: Correlation of molecular aberrations with cytogenetic analysis. The results of the molecular mapping studies were compared with results of cytogenetic analyses and are summarized in Table 2. Rearranged bands were shown in HindIII, EcoRI, and BssHII digests in 13 cytogenetically proven t(4;11) cases and in 1 t(11;19) case (Fig 2A). Therefore, molecular analysis using probes from the breakpoint-clustering region invariably identified the 11q23 aberrations previously shown by cytogenetic analysis. Case 5, diagnosed as t(4;11)(q35;q14-22), had a different cytogenetic breakpoint from the t(4;11)(q21;q23) seen in most t(4;11) cases. Nevertheless, rearrangement of the 11q23 region was shown in this case using both 98.40 and P/S4 probes in all three enzyme digests. These findings indicate that molecular analysis could detect 11q23 rearrangement in case 5 that was not visible cytogenetically. BM samples obtained both at the time of the initial diagnosis and at the time of relapse were available from case 2 that has a three-way translocation involving chromosomes 4, 11, and 14. Analysis of both these samples showed identical rearranged bands in BssHII, HindIII, and EcoRI digests probed with P/S4, 98.40, and 4.2E probes, indicating identical rearrangements in the relapsed as well as in the original leukemic clones (Fig 2A, lanes 2 and 2-R).

Furthermore, seven cases without cytogenetically detectable abnormalities of 11q23 (cases 3, 8, 9, 12, 15, 17, 20), including one normal karyotype (case 20), five cases with inadequate metaphases, and one case for which cytogenetic analysis was not performed, had rearrangements in the 11q23 region by molecular analysis (Fig 2B). Rearrangements in these cases were also mapped to the 11q23 breakpoint clustering region discussed in the previous section and shown in Fig 1.
11q23 molecular abnormalities are strongly correlated with adverse clinical and laboratory features of infant ALL. We compared the observed molecular defects with clinical and laboratory features that have previously been shown to be associated with adverse outcome in infant ALL. Infant ALL cases with 11q23 rearrangements were strongly associated with high leukocyte count compared with cases with germline 11q23. The mean leukocyte count in the group with 11q23 rearrangements was 497,400/μL ± 136,300 (±SE) compared with 33,300/μL ± 9,500 (±SE) in the 11q23 germline group (P < .001). Therefore, 11q23 molecular rearrangements are strongly correlated with hyperleukocytosis.

In addition, 11q23 rearrangements were found in most patients in this study under 6 months of age (17 of 18), in contrast to infants above 6 months where only 4 of 12 had 11q23 rearrangements (P < .001). Lack of CD10 (CALLA) expression was noted in a much higher percentage of infant ALL cases with 11q23 rearrangements (19 of 21) than in cases with germline 11q23 (2 of 9) (P < .001). No significant differences were found in CD24, CD34, or CD9 expression (P = .29, .99, and .99, respectively). The incidence of CNS leukemia was higher in cases with 11q23 rearrangements (11 of 21) when compared to infants with 11q23 germline configurations (2 of 9), but the difference was not statistically significant (P = .23).

11q23 molecular aberrations predict a poor treatment outcome. The aggregate event-free survival in this group of 30 infant ALL cases was 34% at 48 months. This does not differ from the overall results of infant ALL treated with the same protocols that patients of this study are enrolled in. To assess the possible prognostic implication of 11q23 molecular aberrations detected in this group, we retrospectively compared the time to treatment failure in cases with and without 11q23 rearrangements (Fig 3). Our data indicate that infants with 11q23 aberrations were much more likely to suffer early re-...
lapse (N = 15, cases 1 through 9, 11 through 19, and 21) or to fail to enter remission (N = 2, cases 10 and 20) compared to those without 11q23 abnormalities (event-free survival, P < .001, logrank test). The 11q23 abnormality was associated with a 14.4-fold increased risk of an adverse event. Infants whose leukemic cells exhibit rearrangement at 11q23 (N = 21, cases 1 through 21) had a projected event-free survival of 15% (SE, 8.8%) at 48 months. In contrast, molecular genetic analysis defined a group of infant ALL patients (N = 9, cases 22 through 30) with germline 11q23 that showed a projected event-free survival of 80% (SE, 18%) at 48 months (17 to 117 months, median follow-up, 46 months), and case 24 continues in first remission over 117 months (Fig 3). In fact, two patients without 11q23 rearrangements (cases 24 and 28) with CD10 (CALLA) negative ALL are both long-term survivors (over 46 and 117 months, respectively). Patients were treated on successive ALL clinical trials and differences in intensity of therapy cannot be excluded because the relatively small sample size does not permit subgroup analysis.

Age less than 6 months has been reported to be associated with a worse prognosis compared with age 7 to 12 months in infant ALL. This correlation is confirmed in the present study; infant ALL patients less than 6 months of age are significantly more likely to suffer an early relapse or fail to enter remission (14 of 18) compared to those with age 7 to
11q23 abnormally in infant ALL. The P value is indicated. Case numbers are included within the parentheses.

Fig 3. Event-free survival curves in infant ALL with and without 11q23 rearrangement. The analysis used was the Kaplan-Meier procedure and the difference was compared by the logrank test; the P value is indicated. Case numbers are included within the parentheses.

12 months (4 of 12) (P = .02). As mentioned previously, we observed a strong correlation between 11q23 rearrangement and young age. Therefore, molecular rearrangements on 11q23 are tightly associated with young age (1 to 6 months) and predicted a poor treatment outcome. It is worth noting that of the 4 patients aged 7 to 12 months with 11q23 rearrangements, 3 had very short durations of first remission (cases 18, 19, and 20) and that the 1 infant under 6 months without rearranged 11q23 is continuously in remission at 22+ months.

We next investigated whether the location of breakpoints or rearrangements affected treatment outcome in infant ALL cases with 11q23 abnormalities. The location of rearrangements in 20 cases (cases 1 through 18, and 20 through 21; one case [case 19] was not mapped) with respect to the three different EcoRI fragments (see Fig 1) did not influence the duration of first remission using the logrank test (EcoA v EcoB, P = .2; EcoA v EcoC, P = .35; EcoB v EcoC, P = .24, respectively).

DISCUSSION

In the present study, we have identified DNA probes from the 11q23 breakpoint-clustering region that show molecular rearrangements in 21 of 30 (70%) cases of infant ALL selected only by the availability of cryopreserved diagnostic BM samples for study. Using these probes, rearrangements of 11q23 could be identified by molecular analysis in all cases shown by cytogenetic analysis to have 11q23 translocations and in one case with a translocation involving 11q14-22 (case 5). In addition, molecular defects on 11q23 were also found in one case in which no 11q23 abnormalities were detected by cytogenetic analysis, as well as in five cases with insufficient metaphases for complete cytogenetic analysis and also in one case for which karyotype is unavailable. It is worth noting that the five cases with insufficient metaphases (based on our strict criteria) were not found to have detectable cytogenetic 11q23 abnormalities with the material available. Cytogenetic analysis is often complicated by difficulties in obtaining sufficient marrow and the frequent low mitotic index of blast cells in infant leukemia. However, in the present study, molecular analysis could be performed in all samples obtained, many of which had as few as 1 million lymphoblasts for analysis. Therefore, molecular analysis may be preferable to cytogenetics in the future as a diagnostic method to detect 11q23 rearrangement in infant ALL, once probes from this region are more readily available. The high frequency of 11q23 abnormalities in infant ALL is very striking and the incidence in the present molecular study exceeds that previously reported using routine cytogenetic analysis.6,11 These findings suggest that the leukemic cells of infant ALL lacking cytogenetically detectable 11q23 abnormalities not infrequently have 11q23 aberrations that can be identified only by molecular analysis. 11q23 rearrangements in cases without abnormalities detected by cytogenetics might be caused by interstitial exchange, deletion, or local DNA inversion.

We also showed a strong correlation between the presence of 11q23 molecular defects and prognostically adverse clinical factors including age less than 6 months old, a CD10 (CALLA) negative immunophenotype, as well as a high leukocyte count. Our data also suggest that the outcome of infants with ALL may be strongly influenced by the presence or absence of 11q23 molecular defects. Molecular genetic analysis confirms that patients with 11q23 abnormalities have a poor prognosis, as shown previously by cytogenetic analysis.6,9,11,13 Moreover, our results, albeit with a relatively small number of cases analyzed retrospectively, indicate that patients without 11q23 rearrangements had a projected disease-free survival of 80% at 48 months compared with 15% in cases with 11q23 rearrangements. To our knowledge, this is the first molecular prognostic factor that may identify a group of infants with ALL with a good prognosis. Previous cytogenetic studies of infant ALL have been unable to define such a group, likely because some patients had 11q23 abnormalities that were not detectable by cytogenetic analysis but would have been detectable by molecular analysis. We are currently performing a prospective assessment of 11q23 molecular genetic rearrangements in identically treated infants to confirm the prognostic significance of this chromosomal aberration. The poor survival in the 3 of 4 patients with rearranged 11q23 who were in the 7- to 12-month age group suggests that 11q23 rearrangement may be a more important predictor than age for outcome in infant ALL. As more cases are studied, we will be able to investigate the independent prognostic importance of 11q23 molecular rearrangement through multivariate analysis.

The observation that molecular aberrations of 11q23 often involve different partner chromosomes or genetic loci such as 4q13, 4q21, 4q35, and 19q13 suggests that 11q23 rearrangements are the common feature in most cases of ALL in infants. Furthermore, a study of t(11;19)(q23;p13) acute leukemia, including two ALL cases and one case of AML, showed heterogeneity in the breakpoints of chromosome 19p13 among patients with t(11;19), as the chromosome 19 breakpoints were found to be proximal to the insulin receptor (INSR) gene locus in the AML case and distal in the two
ALL cases. The findings argue for the importance of breakpoints at 11q23 in events leading to leukemogenesis in infant ALL. However, a role for chromosome 4 breakpoints in the pathogenesis of t(4;11) acute leukemia is also likely. Because of the consistent phenotypic and clinical features of t(4;11) ALL, it has been proposed that nonrandom breaks on both chromosomes 4 and 11, rather than the breakpoint at 11q23 alone, is the factor associated with the unique age distribution and poorer prognosis in these patients. Our previously reported pulsed-field gel studies are consistent with the hypothesis that the unique clinical and prognostic features of ALL in infants are related to the alteration of genes on both chromosome 11q23 and 4q21. Future studies on chromosome 4q21 are necessary to confirm this hypothesis. A large gene with homology to Drosophila trithorax gene (named MLL or HrX or HTRX-I or ALL-1) has recently been identified that spans the 11q23 breakpoints in t(4;11), t(9;11), and t(11;19) acute leukemia. In this study, the molecular alteration in all cases with 11q23 rearrangements could be mapped to a 15-kb DNA fragment (including EcoA, EcoB, and EcoC) within this gene and most of them were mapped to a 4.6-kb EcoRI fragment (EcoB). These studies suggest that alteration of the human homolog of Drosophila trithorax gene on 11q23 is likely of fundamental importance in the pathogenesis of ALL in infants.

Of interest are previous studies indicating that children with ALL who are treated with epipodophyllotoxins sometimes develop secondary leukemias with 11q23 abnormalities. Epipodophyllotoxins are known to inhibit topoisomerase II and this suggests that the availability of agents in utero or at the time of birth that alter topoisomerase II activity.

Our studies indicate that molecular genetic analysis is an important tool in understanding acute leukemia with 11q23 abnormalities. Furthermore, identifying the molecular defects on 11q23 may be helpful in predicting the clinical course and in planning treatment in infant ALL.

ACKNOWLEDGMENT
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11q23 MOLECULAR REARRANGEMENT IN INFANT ALL


Molecular rearrangements on chromosome 11q23 predominate in infant acute lymphoblastic leukemia and are associated with specific biologic variables and poor outcome

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