

NOTCH1 Mutations in T-Cell Acute Lymphoblastic Leukemia: Prognostic Significance and Implication in Multifactorial Leukemogenesis

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Abstract Purpose: NOTCH signaling pathway is essential in T-cell development and *NOTCH1* mutations are frequently present in T-cell acute lymphoblastic leukemia (T-ALL). To gain insight into its clinical significance, *NOTCH1* mutation was investigated in 77 patients with T-ALL.

Experimental Design: Detection of *NOTCH1* mutation was done using reverse transcription-PCR amplification and direct sequencing, and thereby compared according to the clinical/biological data of the patients.

Results: Thirty-two mutations were identified in 29 patients (with dual mutations in 3 cases), involving not only the heterodimerization and proline/glutamic acid/serine/threonine domains as previously reported but also the transcription activation and ankyrin repeat domains revealed for the first time. These mutations were significantly associated with elevated WBC count at diagnosis and independently linked to short survival time. Interestingly, the statistically significant difference of survival according to *NOTCH1* mutations was only observed in adult patients (>18 years) but not in pediatric patients (≤18 years), possibly due to the relatively good overall response of childhood T-ALL to the current chemotherapy. *NOTCH1* mutations could coexist with *HOX11*, *HOX11L2*, or *SIL-TAL1* expression. The negative effect of *NOTCH1* mutation on prognosis was potentiated by *HOX11L2* but was attenuated by *HOX11*.

Conclusion: *NOTCH1* mutation is an important prognostic marker in T-ALL and its predictive value could be even further increased if coevaluated with other T-cell-related regulatory genes. NOTCH pathway thus acts combinatorially with oncogenic transcriptional factors on T-ALL pathogenesis.

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T-cell acute lymphoblastic leukemia (T-ALL) is a malignant disease of thymocytes, accounting for 10% to 15% of pediatric and 25% of adult ALL cases. Although the outcome of T-ALL has improved dramatically with current therapy, significant challenges remain, including understanding of the factors that contribute to the malignant behavior of these leukemic cells and developing subsequently optimal biomarkers to predict prognosis in T-ALL patients (1).

Oncogenic transcription factors are often aberrantly expressed in T-ALL and related to leukemia development (2). Initially identified from chromosomal translocations where fusion genes are generated, disruption of normal expression control of transcription factors may present irrespectively to these rearrangements and lead to dysregulation of differentiation, self-renewal, and proliferation of hematopoietic stem cells.

NOTCH signaling controls T-cell fate decisions and regulates normal T-cell development (3). *NOTCH1*, as an important receptor of NOTCH family, was first discovered through its involvement in a t(7;9)(q34;q34) chromosomal translocation seen in T-ALL and was subsequently proved essential for T-cell leukemogenesis (4, 5). Mice reconstituted with hematopoietic

progenitor cells expressing activated *NOTCH1* allele develop T-cell leukemia (5, 6) whereas blockade of NOTCH pathway suppresses the growth and survival of *NOTCH1*-transformed T-ALL cells (7). Recently, gain-of-function *NOTCH1* mutations were reported in T-ALL patients, confirming the pathogenetic role of *NOTCH1* activation on T-ALL (8). However, clinical significance of *NOTCH1* mutations, especially their effect on prognosis, remains to be investigated.

In this study, we evaluate the clinical significance and prognostic value of the *NOTCH1* mutation in T-ALL patients. Moreover, because leukemia development involves multiple processes combining abnormality at both transcriptional regulation and cytoplasmic signaling levels, as already shown in acute and chronic myeloid leukemia models (9, 10), we raised the same question for *NOTCH1* mutations and other oncogenic transcription factors in T-ALL. By reverse transcription-PCR, the expression of three additional oncogenes specifically involved in T-ALL, homeobox genes *HOX11* and *HOX11L2*, as well as *SIL-TAL1* fusion gene (11), were assessed to investigate their possible association with *NOTCH1* mutation on disease outcome in T-ALL.

Patients and Methods

Patients. Seventy-seven newly diagnosed T-ALL patients (61 male and 16 female) who achieved complete remission after treatment and subsequently possessed follow-up data were enrolled in this study, including 53 pediatric patients (≤ 18 years; median, 10 years) and 24 adult patients (> 18 years; median, 24 years). The main characteristics of these patients are summarized in Table 1. T-cell lineage was defined by the presence of the T-cell antigen CD3 either on the cell surface or in the cytoplasm, with at least two of the B-cell markers (CD19, CD20, CD22, CD79a) and three of the myeloid markers (CD11b, CD13, CD33, myeloperoxidase) tested negative.

For adult patients, the induction course was VDCP regimen administered over a 4-week period (vincristine 1.5 mg/m², days 1, 8, 15, and 22; daunorubicin 30 mg/m² or idarubicin 9 mg/m², days 1-3; cyclophosphamide 800 mg/m², days 1 and 8; prednisone 60 mg/m², days 1-28). A supplement dose of 6,000 IU/m² L-asparaginase every other day was added on days 15 to 29 when bone marrow blasts persisted $> 5\%$ on day 14. The patients then received a course combining cytarabine (1 g/m² every 12 hours, days 1-4) with mitoxantrone (10 mg/m², days 3-5).

For pediatric patients, the conventional protocol for remission induction was VDLP regimen (vincristine 1.5 mg/m², days 1, 8, 15, and 22; daunorubicin 30 mg/m² or idarubicin 9 mg/m², days 8-10; L-asparaginase 6,000 IU/m² every other day, days 9-23; prednisone 60 mg/m² per day, days 1-28), followed by one cycle of cyclophosphamide (800 g/m², day 1) + cytarabine (1 g/m² every 12 hours, days 2-4) + 6-mercaptopurine (75 mg/m², days 1-7) regimen.

The consolidation therapy of all the patients included three cycles of high-dose methotrexate regimen (methotrexate 3-5 g/m² for 1 day every 10 days, three courses continuously), one cycle of VDLEx regimen (same dosage of vincristine and daunorubicin/idarubicin on days 1 and 8, with L-asparaginase 6,000 IU/m² every other day on days 2-12 and dexamethasone 6 mg/m² per day on days 1-14), followed by one cycle of EA regimen (etoposide, 300 mg/m², cytarabine, 300 mg/m², days 1, 4, and 7). The maintenance therapy was 6-mercaptopurine 75 mg/m² per day for 3 weeks and methotrexate 20 mg/m² per week, thrice, combined with intermittent intensive chemotherapy using VDLEx, EA, or high-dose methotrexate regimen. The whole therapy was stopped at 3 years after complete remission.

Seventy patients with B-ALL and 102 healthy volunteers were referred as controls. Approval for these studies was obtained from the

Table 1. Patient characteristics and *NOTCH1* mutation

| Patient characteristics | <i>NOTCH1</i> mutation (+) | <i>NOTCH1</i> mutation (-) | P |
|------------------------------------|----------------------------|----------------------------|--------|
| Gender | | | |
| Male | 26 | 35 | 0.0794 |
| Female | 3 | 13 | |
| Age (y) | | | |
| ≤ 18 | 18 | 35 | 0.3194 |
| > 18 | 11 | 13 | |
| WBC count | | | |
| $< 10 \times 10^9/L$ | 3 | 15 | 0.0357 |
| $> 10 \times 10^9/L$ | 26 | 33 | |
| Hepatosplenomegaly | | | |
| Yes | 11 | 21 | 0.6156 |
| No | 18 | 27 | |
| Mediastinum involvement | | | |
| Yes | 12 | 12 | 0.1327 |
| No | 17 | 36 | |
| Central nervous system involvement | | | |
| Yes | 9 | 6 | 0.0673 |
| No | 20 | 42 | |
| <i>HOX11</i> expression | | | |
| <i>HOX11</i> (+) | 8 | 14 | 0.8818 |
| <i>HOX11</i> (-) | 21 | 34 | |
| <i>HOX11L2</i> expression | | | |
| <i>HOX11L2</i> (+) | 6 | 8 | 0.6574 |
| <i>HOX11L2</i> (-) | 23 | 40 | |
| <i>SIL-TAL1</i> expression | | | |
| <i>SIL-TAL1</i> (+) | 3 | 7 | 0.5919 |
| <i>SIL-TAL1</i> (-) | 26 | 41 | |

University and Institutional Review Boards. All patients gave accordingly their informed consent.

RNA extraction and reverse transcription-PCR analysis. For each patient, bone marrow sample was collected at diagnosis and mononuclear cells were enriched by density-gradient centrifugation with Ficoll solution. As assessed morphologically and cytochemically, all samples consisted of $> 85\%$ leukemic blasts. Total RNA was extracted using TRIzol agent (Invitrogen, Carlsbad, CA). First-strand cDNA was synthesized from 1 μ g total RNA using Superscript II reverse transcriptase (Invitrogen) and random hexamers according to the instructions of the manufacturer.

The transmembrane segment and intracellular region of *NOTCH1* were amplified using standard protocol with seven pairs of primers: *NOTCH1*-1 (F: 5'-CCTGGAAGAACTGCACGCA-3' and R: 5'-CAATCTCCAGGTAGACGATGGAG-3') for the NH₂-terminal region of the heterodimerization domain (HD-N); *NOTCH1*-2 (F: 5'-ATCTTCCCC-TACTACGGCCG-3' and R: 5'-AAGAACAGAAGCACAAGGGCG-3') for the COOH-terminal region of the HD (HD-C); *NOTCH1*-3 (F: 5'-TG-CACTTCATGTACGTGGCG-3' and R: 5'-CCTGGTAGATGAAGTCGGA-GATG-3') for the RAM domain and the N-region of the ankyrin repeat (ANK) domain; *NOTCH1*-4 (F: 5'-CAACAGCCGAGGAAGAGGAGG-3' and R: 5'-TGTCGGGTTGGCAAAGTG-3') for the middle part of the ANK domain; *NOTCH1*-5 (F: 5'-CGCAGTTGTCTCCTGAAGAAC-3' and R: 5'-ACGGACGGAGACTGCTGGAA-3') for the C-region of ANK domain and one part of the transcription activation domain (TAD); *NOTCH1*-6 (F: 5'-TGGAGTACCCCATGGCTAC-3' and R: 5'-CTCG-GCTCTCCACTCAGGAA-3') for the rest of the TAD domain and one part of the proline/glutamic acid/serine/threonine (PEST) domain; and

NOTCH1-7 (F: 5'-GCTGCACAGTAGCCTTGCTG-3' and R: 5'-GCGCG-CCGTTTACTTGAAG-3') for the rest of the PEST domain.

HOX11, *HOX11L2*, and *SIL-TAL1* expressions were assessed using the corresponding primers by reverse transcription-PCR: *HOX11* (F: 5'-TGGATGGAGAGTAACCGCAGAT-3' and R: 5'-AGGTACTTCTGG-CGGTGGAA-3'); *HOX11L2* (F: 5'-CGCCAAGTCCCTCAAAATGA-3' and R: 5'-CGGGAACCTTGGAACTATCCT-3'); and *SIL-TAL1* (F: 5'-CCGCG-ACCCCAACGT-3' and R: 5'-CTCATTTCTTGCTGAGCTTCTTGTC-3').

NOTCH1 mutation detection. The resultant PCR products were purified on Qiagen columns (Qiagen, Inc., Valencia, CA) and sequenced by *NOTCH1* primers on ABI Prism 3700 DNA Analyzer using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA).

For further confirmation of the insertion and deletion mutations, the purified PCR products were ligated into pGEM-T Easy Vector Systems (Promega Corporation, Madison, WI) and used to transform DH5 α *E. coli* cells. On agarose gel with ampicillin, individual colonies were screened by isopropyl- β -thio- β -D-galactopyranoside and 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside, quantified on electrophoresis, and sequenced by T7 and SP6 primers following the same protocol.

Statistical analyses. Patient characteristics were compared using χ^2 analysis and Fisher's exact test. Overall survival was measured from the date of diagnosis to the date of death or last follow-up through January 31, 2006. Relapse-free survival was calculated from the date of complete remission to the date of first relapse. Survival functions were estimated using the Kaplan-Meier method and compared by the log-rank test. Multivariate survival analysis was done using a Cox regression model. $P < 0.05$ was considered statistically significant. All statistical analyses were evaluated using SAS 8.2 software (SAS Institute, Inc., Cary, NC).

Results

NOTCH1 mutations in T-ALL. Through direct sequencing and elimination of a number of single-nucleotide polymorphisms by typing 102 healthy volunteers, 32 *NOTCH1* mutations were revealed in 29 of the 77 patients, involving HD (23 cases, 29.9%), PEST (5 cases, 6.5%), TAD (3 cases, 3.9%),

and ANK regions (1 case, 1.3%). None of these mutations was found in 70 patients with B-ALL and 102 healthy volunteers. Interestingly, mutation was absent from remission bone marrow sample obtained from one patient whose T-ALL harbored *NOTCH1* mutations in HD sequence (Fig. 1A, I).

All the *NOTCH1* mutations detected were listed in Table 2 and illustrated in Fig. 1B. The mutations occurring in HD included 13 point mutations, 5 insertions, 2 deletions, 2 deletion/insertions, and 1 insertion + duplication. These mostly fell in HD-N (16 cases) whereas mutations of HD-C were also identified (7 cases). The HD-N mutations were all clustered in a "hotspot" spanning residues 1,575 to 1,610 and included, more frequently, three L-to-P missense mutations on residue 1,586 (nos. 3-5) and four insertions from residue 1,607 (nos. 12-15). Overall, seven new types of mutation were found on the sites previously reported (ref. 8; nos. 6, 10, and 12-16). In HD-C, with the missense mutations on residue 1679 (nos. 18-20) most often observed, one new type of insertion from residue 1,677 (no. 17) and one insertion on an undescribed site from residue 1,741 (no. 23) were identified. *NOTCH1* mutations within HD region coexisted with mutations of PEST domain (one case, no. 3) and TAD region (two cases, nos. 17 and 20).

The PEST mutations were less frequently detected, including three point mutations, one insertion, and one deletion/insertion, all of which were newly identified. They induced either a substitution in reading frame (no. 27) or premature stop codons (nos. 3, 26, 28, and 29; Fig. 1A, IV and VI).

In addition to HD and PEST sequence, mutation detection was done on RAM, ANK, and TAD regions. One N-to-I mutation on residue 1,900 was found in ANK domain (no. 24; Fig. 1A, II). Two frameshift mutations on residues 2,262 and 2,330 (nos. 20 and 17) inducing premature stop codons, as well as one point mutation (P-to-S on residue 2,272, no. 25) of *NOTCH1*, were found in TAD region (Fig. 1A, III and V). These mutations were not reported previously.

Fig. 1. *NOTCH1* mutations in T-ALL. *A*, *NOTCH1* mutation of HD-C (L1679P; *I*) presented at diagnosis became undetectable after complete remission. A point mutation (N1900I; *II*) was found in ANK domain. In TAD region, both point mutation (P2272S; *III*) and frameshift mutation (G2262Xfs; *V*) were observed. In PEST domain, a premature stop of *NOTCH1* was induced either by point mutation (Q2407X; *IV*) or insertion (S2423X; *VI*). Black arrows, site of point mutation; asterisks, stop codons. *B*, schematic representation of human *NOTCH1* showing the distribution of mutations in T-ALL. White arrows, mutations first described in our study; black arrows, previously reported mutations.

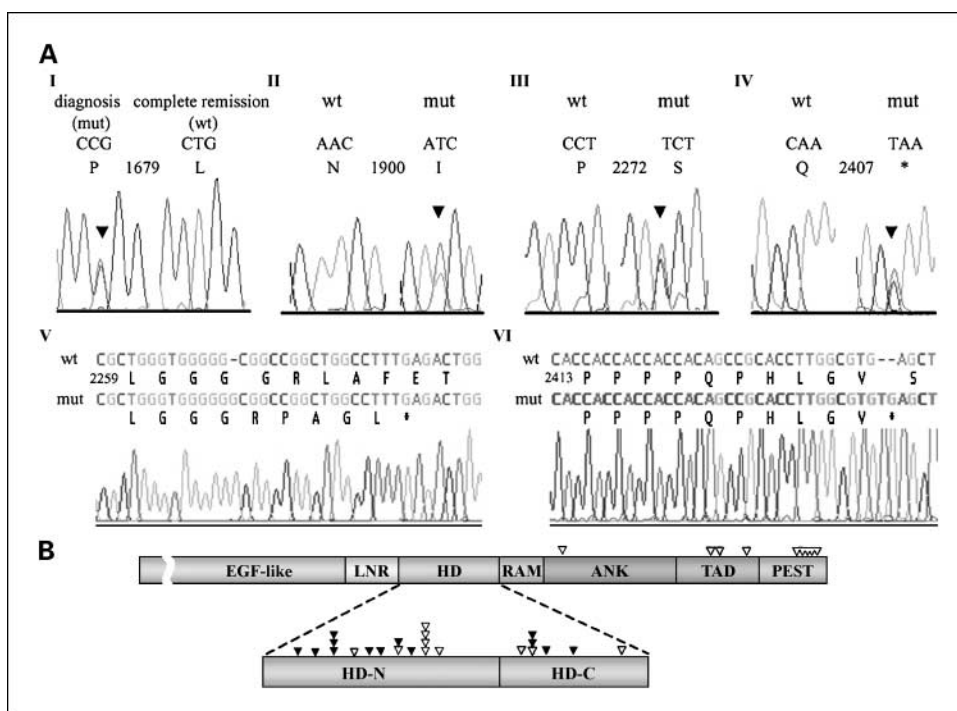


Table 2. NOTCH1 mutations in T-ALL patients

| DOM | Case no. | Classes | Type | Amino acids involved | Status | |
|------|----------|---------|---|-----------------------------------|------------------|---|
| HD-N | 1 | PM | 4724 T>C | L1575P | R | |
| | 2 | DEL | 4735.4737 del GTG | V1579 del | R | |
| | 3-5 | PM | 4757 T>C | L1586P | R | |
| | 6 | DEL/INS | 4771.4788 delins CTTCTGGGGGGG | F1591.E1596 delins LLGG | N | |
| | 7 | PM | 4778 T>C | F1593S | R | |
| | 8 | PM | 4781 T>C | L1594P | R | |
| | 9 | PM | 4796 G>C | R1599P | R | |
| | 10 | INS | 4795.4796 ins AAA | R1599 delins QS | N | |
| | 11 | PM | 4802 T>C | L1601P | R | |
| | 12 | INS | 4820.4821 ins GGACCC | F1607 delins LDP | N | |
| | 13 | INS | 4820.4821 ins GGGGAC | F1607 delins LGT | N | |
| | 14 | INS | 4820.4821 ins GAACCCCTATC | F1607 delins LNPLS | N | |
| | 15 | DEL/INS | 4821 delins ACCCCCACACCCT | F1607 delins LPPHP | N | |
| | 16 | DEL | 4829.4903 del | D1610.R1634 del | N | |
| | HD-C | 17 | INS | 5029.5030 ins GAATCG | V1677 delins GIV | N |
| | | 18-19 | PM | 5036 T>C | L1679P | R |
| 20 | | PM | 5036 T>A | L1679Q | N | |
| 21 | | PM | 5042 T>A | I1681N | R | |
| 22 | | PM | 5104 G>C | A1702P | R | |
| 23 | | INS+DUP | [5222.5223 ins AATGAAGCT+5178.5222 dup] | [1741.1742 ins MKL+1727.1741 dup] | N | |
| ANK | 24 | PM | 5699 A>T | N1900I | N | |
| TAD | 20 | INS | 6785.6786 ins G | G2262Xfs | N | |
| | 25 | PM | 6814 C>T | P2272S | N | |
| | 17 | INS | 6987.6988 ins G | S2330Xfs | N | |
| PEST | 26 | PM | 7219 C>T | Q2407X | N | |
| | 27 | PM | 7264 G>A | V2422M | N | |
| | 3 | INS | 7266.7267 insTG | S2423X | N | |
| | 28 | PM | 7271 C>A | S2424X | N | |
| | 29 | DEL/INS | 7478.7505 delinsTCTCCTGAAGAGG | S2493Xfs | N | |

Abbreviations: DOM, domain of mutations; PM, point mutation; DEL, deletion; INS, insertion; DUP, duplication; R, previously reported; N, previously undescribed.

Clinical and prognostic significance associated with NOTCH1 mutation. As shown in Table 1, among nine clinical and biological variables, NOTCH1 mutation was more frequent in the patients with WBC count $>10 \times 10^9/L$ than those with normal level [26 of 59 (44.1%) versus 3 of 18 (16.7%), $P = 0.0357$]. When the cutoff was elevated to $50 \times 10^9/L$ or $100 \times 10^9/L$, although a similar increase of NOTCH1 mutation was found [18 of 41 (43.9%) versus 11 of 36 (30.6%) and 14 of 31 (45.2%) versus 15 of 46 (32.6%), respectively], they failed to reach statistical significance ($P = 0.2278$ and $P = 0.2649$, respectively).

Of the 77 patients, the 3-year relapse-free survival and overall survival rate (\pm SE percentage) were respectively 47.2% ($\pm 6.4\%$) and 54.6% ($\pm 6.7\%$), with median relapse-free survival at 24 months and median overall survival not reached at the time of last follow-up. Poor relapse-free survival and overall survival rate were correlated with NOTCH1 mutation. The 3-year relapse-free survival and overall survival rate (\pm SE percentage) for patients with NOTCH1 mutation were 28.8% ($\pm 8.8\%$) and 31.8% ($\pm 9.5\%$), significantly shorter than patients without mutation [59.8% ($\pm 8.4\%$) and 71.7% ($\pm 7.9\%$); $P = 0.0053$ (Fig. 2A) and $P = 0.0026$, respectively].

Multivariate analysis revealed that NOTCH1 mutation independently correlated with inferior relapse-free survival [$P = 0.0084$; hazard ratio, 2.4; 95% confidence interval (95% CI), 1.3-4.6] and overall survival ($P = 0.0065$; hazard ratio, 2.9; 95% CI, 1.3-6.1) in T-ALL patients. Other variables entered with NOTCH1 mutation in the Cox regression model were age and WBC count.

Because age was also an independent negative indicator both on relapse-free survival ($P = 0.0369$; hazard ratio, 2.1; 95% CI, 1.0-4.0) and overall survival ($P = 0.0221$; hazard ratio, 2.4; 95% CI, 1.1-5.1), we further divided the 77 patients into two groups according to the age at diagnosis. In the pediatric group, although decreased survival did present in patients displaying NOTCH1 mutation, no significant difference was obtained [$P = 0.2763$ for relapse-free survival (Fig. 2B); $P = 0.1712$ for overall survival]. In the adult group, patients positive for NOTCH1 mutation had a clearly worse prognosis than those negative for mutation [$P = 0.0015$ for relapse-free survival (Fig. 2C); $P = 0.0041$ for overall survival]. Moreover, NOTCH1 mutation was still an independent adverse prognostic factor in this group both for relapse-free survival ($P = 0.0049$; hazard ratio, 5.4; 95% CI, 1.7-17.5) and for overall survival ($P = 0.0106$; hazard ratio, 5.5; 95% CI, 1.5-15.3).

Coexistence of NOTCH1 mutations with oncogenic transcription factors and their combined effect on disease outcome. All the patients were studied for *HOX11*, *HOX11L2* expression, and *SIL-TAL1* fusion gene. The *NOTCH1* mutations were seen associated with expression of *HOX11* (8 of 22 cases, 36.4%), *HOX11L2* (6 of 14, 42.9%), and *SIL-TAL1* (3 of 10, 30.0%). However, no significant relation was observed between *NOTCH1* mutation and incidence of *HOX11*, *HOX11L2*, and *SIL-TAL1* expression (Table 1).

In all, *HOX11L2* expression indicated shorter relapse-free survival ($P = 0.0052$) but no difference in overall survival ($P = 0.0649$). When combining *NOTCH1* mutation with *HOX11L2* expression, significantly worse relapse-free survival and overall survival were observed in the patients positive for *NOTCH1* mutation and *HOX11L2* expression, compared with those negative for both factors [$P < 0.0001$ for relapse-free survival (Fig. 3A); $P = 0.0102$ for overall survival, respectively].

HOX11 expression was associated with longer relapse-free survival and overall survival ($P = 0.0497$ and $P = 0.0246$). Distinguished according to *NOTCH1* mutation and *HOX11* expression, as shown in Fig. 3B, patients with *NOTCH1* mutation were stratified into two outcomes depending on the status of *HOX11* expression. Favorable prognosis was noted in patients additionally positive for *HOX11*, in contrast to those negative for *HOX11* who presented with short survival ($P = 0.0121$ for relapse-free survival (Fig. 3B); $P = 0.0163$ for overall survival).

These prognostic effects of the genetic subgroup were not significant when further divided according to the age distribution, probably because of the limited number of patients included in each group.

Discussion

NOTCH1 expression has been implicated in promoting T lineage choice from common lymphoid progenitors and its mutations are frequently present in T-ALLs (3, 8). In our study, using reverse transcription-PCR amplification and direct sequencing, we showed *NOTCH1* mutations both in pediatric and adult T-ALL patients. These mutations were not observed in patients with B-ALL, indicating that they are restricted to T-ALL (8). Although the incidence of *NOTCH1* mutation is relatively lower than that previously reported (8, 12), by the same technique, the confirmation of previously described mutations with the discovery of new ones suggested that racial difference might exist between Asian and Western countries (13). This needs further study on a larger series of patients. Of note, its disappearance after remission revealed that *NOTCH1* mutation acquired within the malignant clones could thus be served as a biomarker for disease surveillance.

The *NOTCH1* mutations were mainly observed in the HD and PEST domains. Responsible for maintaining stable association between extracellular and transmembrane subunit (14), the

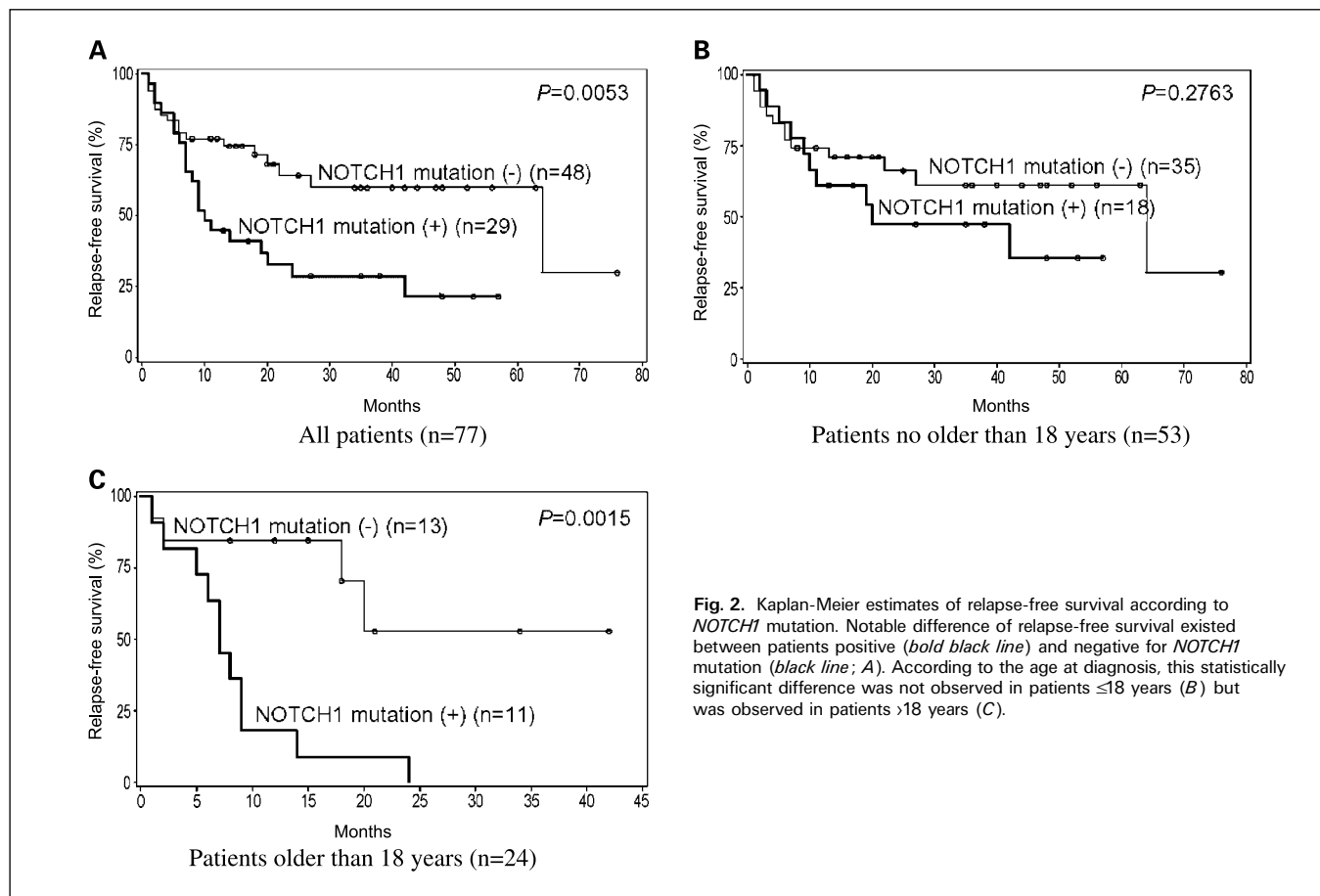


Fig. 2. Kaplan-Meier estimates of relapse-free survival according to *NOTCH1* mutation. Notable difference of relapse-free survival existed between patients positive (**bold black line**) and negative for *NOTCH1* mutation (**black line**; A). According to the age at diagnosis, this statistically significant difference was not observed in patients ≤ 18 years (B) but was observed in patients >18 years (C).

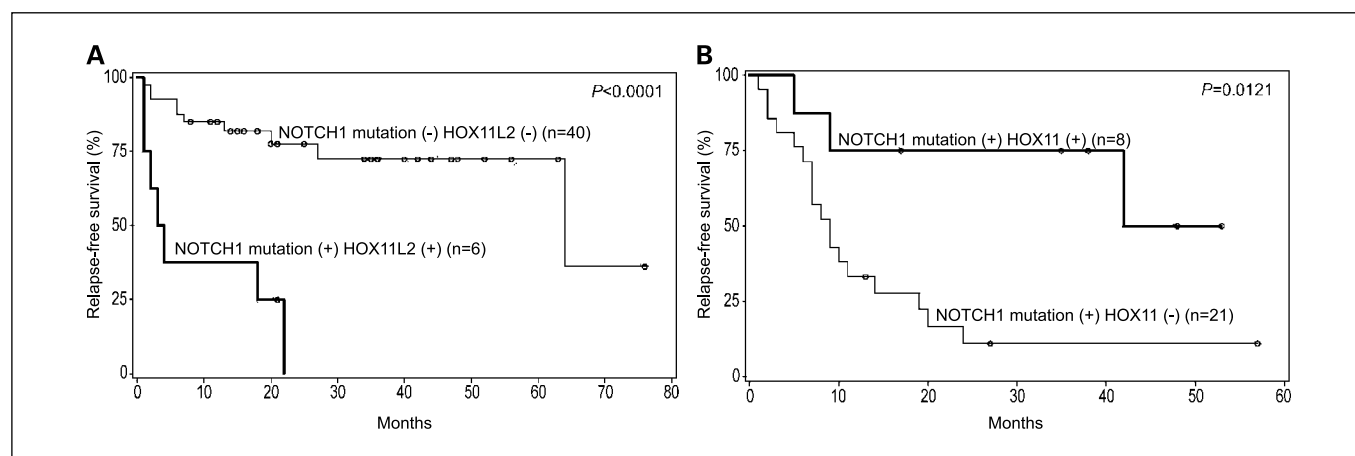


Fig. 3. Kaplan-Meier estimates of relapse-free survival according to *NOTCH1* mutation and oncogenic transcription factors expression. Compared with *NOTCH1* mutation (–) *HOX11L2* (–) cases (black line), significantly reduced relapse-free survival was seen in *NOTCH1* mutation (+) *HOX11L2* (+) patients (bold black line; A). In contrast, *NOTCH1* mutation (+) patients showed a different disease outcome according to *HOX11* expression (B): *NOTCH1* mutation (+) *HOX11* (–) cases (black line) presented with poor prognosis whereas *NOTCH1* mutation (+) *HOX11* (+) patients (bold black line) presented with good prognosis.

mutation within HD-N may destabilize the subunit interaction, resulting in constitutive *NOTCH1* activation and subsequent cell transformation (15, 16). Moreover, mutations detected in the HD-C sequence could affect the proteolytic sites of furin-like convertase from residue 1,675 to 1,682 and S3 cleavage site from residue 1,743 to 1,744, and thus perturb the processing of the *NOTCH1* receptor (17–20). The PEST region regulates protein turnover by targeting proteins to the ubiquitin-proteasome complex for subsequent degradation (21). The deletions of its COOH-terminal sequences enhance *NOTCH1* intracellular signaling and have been previously reported in murine T-ALL models, presaging the detection of PEST mutations having the similar structural consequences in human T-ALL (16, 22).

In addition to HD and PEST domains, mutations of TAD and ANK domains were identified for the first time in this series of T-ALL. Functionally, ANK interacts with downstream transcription factors, displacing corepressors, and the TAD serves to recruit coactivator molecules (23). Consistent with a previous report in mice model that both domains are required for T-cell leukemogenesis (24), these mutations could also play a role in T-ALL development.

Frequently occurring in T-ALL, *NOTCH1* mutation relates to clinical variable and translates into differences in disease outcome in T-ALL patients. Constitutive NOTCH signaling was reported to be important to maintain T-ALL growth and survival *in vitro* (7). *NOTCH1* is overexpressed in T cell–derived tumor cells of anaplastic large cell lymphoma and its activation accelerates the growth and inhibits the apoptosis of the lymphoma cells (25). This may explain that patients positive for *NOTCH1* mutation displayed increased WBC count. However, it failed to reflect an extremely high proliferative status of the leukemic clone, which could possibly need a different and/or additional aberration. In clinical settings, a direct link has been found in multiple myeloma and lymphoma between activation of *NOTCH1* and protection of tumor cells from apoptosis induced by chemotherapeutic agents or arsenite (25, 26), which could be related to poor disease outcome. In solid tumors, a high level of *NOTCH1* was associated with advanced

stage, metastasis, and poor prognosis in breast cancer (27). The fact that *NOTCH1* mutations were correlated with decreased survival time in T-ALL patients further indicates the importance of NOTCH signaling abnormality in disease progression.

Interestingly, the adverse prognostic effect of *NOTCH1* mutation relied on the age of the patients. Because pediatric patients respond well to current therapy, the significance of molecular biomarker could be weakened by the relatively good prognosis in pediatric group and no significant superiority of survival time was observed in patients without *NOTCH1* mutation. However, in adult patients, who were known to be less frequently cured (1), *NOTCH1* mutation was independently correlated with poor disease outcome and could be considered as a negative prognostic factor.

NOTCH1 mutations participate in T-ALL formation in *E2A-PBX1* or *C-MYC* transgenic mice (16, 22). This capacity of *NOTCH1* to cooperate with other genes in mice models is reminiscent of the association of *NOTCH1* mutations with many molecular subtypes of human T-ALL. Point mutations, as well as insertions and deletions described above, could occur in multipotent hematopoietic progenitors, which normally express *NOTCH1* (28). These aberrations would be predicted to induce daughter cells to adopt a T-cell fate and thereby increase the pool of cells at risk for additional leukemogenic events, including the overexpression of other critical transcription factors.

NOTCH signaling controls the generation and differentiation of early T lineage progenitors (29), which oncogenic transcription factor *HOX11* could possibly act on (30). *HOX11L2* is an orphan homeobox factor very similar to *HOX11* and its ectopic expression reinforces the role of homeobox transcription factors on T-cell leukemogenesis (31). Clinically, *HOX11L2* expression confers a worse response to treatment (30, 32) whereas *HOX11* activation is significantly related to a favorable prognosis in T-ALL (11, 33). In our study, patients with *NOTCH1* mutations had dual outcome in accordance with their expression for *HOX11L2* and *HOX11*: patients additionally positive for *HOX11L2* expression deteriorated quickly whereas those with *HOX11* expression showed prolonged survival. This

may reflect that *NOTCH1*-induced transformation of hematopoietic lineages requires the collaborative action of additional cellular oncogenes.

In conclusion, *NOTCH1* mutations were present in patients with T-ALL and indicated poor prognosis. Cooperatively with other oncogenic transcription factors, *NOTCH1* mutations participate in the pathogenesis of T-ALL, providing a promising

rationale for targeted therapies that interfere with NOTCH signaling pathway.

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Correction: Article on *NOTCH1* Mutations in T-Cell Acute Lymphoblastic Leukemia

In the article on *NOTCH1* mutations in the May 15, 2006 issue of *Clinical Cancer Research*, in the *Patients* section, the dosages for adult patients of the high-dose methotrexate and EA regimens were inadvertently omitted. In adult patients, the dosage for the high-dose methotrexate regimen is 1.5 to 2 g/m² methotrexate for 1 day every 4 weeks and 100 mg/m² etoposide for the EA regimen.

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NOTCH1 Mutations in T-Cell Acute Lymphoblastic Leukemia: Prognostic Significance and Implication in Multifactorial Leukemogenesis

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Clin Cancer Res 2006;12:3043-3049.

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