NOTCH1 mutations are common in T-lineage acute lymphoblastic leukemia (T-ALL). Twin studies and retrospective screening of neonatal blood spots provide evidence that fusion genes and other chromosomal abnormalities associated with pediatric leukemias can originate prenatally. Whether this is also the case for NOTCH1 mutations is unknown. Eleven cases of T-ALL were screened for NOTCH1 mutations and 4 (36%) had mutations in either the heterodimerization (HD) or proline glutamic acid/serine/threonine (PEST) domains. Of these 4, 3 could be amplified by mutation-specific polymerase chain reaction primers. In one of these 3, with the highest sensitivity, NOTCH1 mutation was detected in neonatal blood spots. In this patient, the blood spot was negative for SIL-TAL1 fusion, present concomitant with NOTCH1 mutation, in the diagnostic sample. We conclude that NOTCH1 can be an early or initiating event in T-ALL arising prenatally, to be complemented by a postnatal SIL-TAL1 fusion. (Blood. 2008;111:376-378)
10, and 11) had mutations (confirmed by cloning and sequencing) in either HD (MGT01 and 09) or PEST domains (MGT10 and 11). Three involved insertions of unrelated short sequences and one had a duplication of a relatively long sequence from within NOTCH1 (Table 1). Only one patient sample (MGT01) of the eleven had a SIL-TAL1 fusion. This was a type 1 fusion (data not shown).

PCR primers were designed based on the sequences of patient-specific mutations and specificity and sensitivity were assayed with diagnostic DNA samples and cloning vectors with mutated sequences by serial dilutions. In one patient sample (MTG10), no primer set worked well, therefore samples from only 3 (MTG01, MTG09 and MTG11) with NOTCH1 mutations were further analyzed. With the patient-specific primer sets, positive PCR products were obtained only in corresponding patient DNA showing specificity of the primers (Figure 1A). Sensitivity of mutation-specific primers was assayed by serially diluting diagnostic DNA showing specificity and sensitivity were assayed with SIL-TAL1 (Table 1). Only one patient sample (MGT01) of the eleven had a duplication of a relatively long sequence from within the region of HD-N (MGT01 and MGT09), which may reflect the fact that the DNA was derived from a bone marrow sample that was substantially hemidiluted.

Excised blood spot segments were placed directly into PCR reactions with Ampdirect Plus to maximize the opportunity to amplify rare sequences and at least 16 segments of each spot were analyzed. Normal NOTCH1 genomic sequences corresponding to the site of mutation on the region of HD-N (MGT01 and MTG09) and PEST (MGT11) were amplified as PCR controls and all segments (at least 8 segments in each patient) examined showed positive amplification (data not shown), indicating DNA on the Guthrie cards was intact.

Of the 3 patient samples studied, one, MGT01, showed unambiguous amplification of specific mutated sequence on several segments of blood spots (Figure 1C). In total, 20 pieces of the Guthrie card were subjected to PCR amplification and 11 were positive. Several of the positive bands were purified and sequenced and all had the patient-specific HD mutation (Table 1). A total of 20 pieces of control blood spots from the Guthrie cards of nonleukemic children were examined as additional controls with the MGT01 mutation-specific primer set. No amplification bands were observed. Patient MGT01 also had the SIL-TAL1 gene fusion. Sixteen fragments of blood spots for patient MGT01 were analyzed for the presence of the specific SIL-TAL1 fusion sequence but no PCR product was observed after 2 rounds of PCR amplification (70 cycles of PCR in total; Figure 1D).

Patient MGT01’s blood spots, positive for NOTCH1 mutation, were the only ones for which we obtained sensitivity comparable

### Table 1. Details of NOTCH1 mutations in 4 cases of T-ALL

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age at diagnosis</th>
<th>Mutations in NOTCH1</th>
<th>SIL-TAL1</th>
</tr>
</thead>
<tbody>
<tr>
<td>MGT01</td>
<td>6 y 10 mon</td>
<td>HD: 4894ins(-TCTTACGAGAAACGAGCAG-3')</td>
<td>+ (Type I)</td>
</tr>
<tr>
<td>MGT09</td>
<td>9 y 1 mon</td>
<td>HD: 4894ins(-TCTTTGTGCGCCAG-3')</td>
<td>-</td>
</tr>
<tr>
<td>MGT10</td>
<td>4 y 7 mon</td>
<td>PEST: 7123ins(-GCCCTCCGTGAGCAGTAGGATGAGGAGGCCCTCC-)1</td>
<td>-</td>
</tr>
<tr>
<td>MGT11</td>
<td>5 y 6 mon</td>
<td>PEST: 7403ins(-AGACTGCACTGCA-)</td>
<td>-</td>
</tr>
</tbody>
</table>

Sequences of patient-specific insertional mutation are underlined. + indicates present, - not present.

<sup>a</sup>dup(7097-7122).

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**Figure 1. Detection of NOTCH1 mutation via PCR.**

(A) Specificity of PCR primers for patient-specific mutations was examined by PCR with diagnostic DNA samples of T-ALL patients (MGT01 to MGT11). Primers used for the PCR are shown with the panels. Two rounds of PCR were necessary to obtain a clear amplification band in patient MGT09, possibly because of a low number of leukemic cells. (B) Sensitivity assay of mutation-specific primers. Serially diluted diagnostic DNA was subjected to PCR amplification with Ampdirect Plus in 40 cycles of PCR reaction. F indicates forward and r, reverse PCR primers for mut, mutation. Sequences of the primers for specific mutation are shown. Of 8 MGT01 blood spot pieces, 5 showed positive bands of which 2 were very faint and became clear after seminested PCR amplification (data not shown). All slices from blood spots of MTG09 and MGT11 were negative for their respective NOTCH1 mutation. At least 16 pieces of blood spots, in total, were examined in each patient. C indicates control blood spot without NOTCH1 mutation. (D) Results of SIL-TAL1 amplification on the MGT01 blood spot. Sixteen pieces were examined in total and all were negative, as shown here for 8 slices, even after 2 rounds of PCR amplification.
with that achieved and required with leukemia fusion genes that register positive in blood spots – approximately 100 cells.2,4,7

Negative results in neonatal blood spot screening are uninterpretable but tend to underestimate the frequency of prenatally-initiated leukemias.2 This is particularly so when, as in the present study with NOTCH1 mutation, levels of sensitivity achievable with subtle sequence mutations, while maintaining specificity, are very modest compared with fusion genes.4,7

No general conclusion can be drawn about the usual timing of this common genetic abnormality in T-ALL, but these data indicate that NOTCH1 mutation can occur prenatally as an early or possibly initiating event in T-cell leukemogenesis. This interpretation accords with the view that NOTCH1 regulates self-renewal properties of stem cells or progenitors16,17 and can initiate T-cell leukemogenesis as a transgene in mice18 or zebra fish.19 In animal models of T-ALL, secondary NOTCH1 mutations have been detected in leukemias initiated by other genetic changes.20,21 As in this particular positive case, MGT01, the concurrent diagnostic SIL-TAL1 fusion sequence was absent from the neonatal blood spot (with a sensitivity threshold of ∼10 cells/mutation copies; Figure 1B), then it is likely that the latter was a secondary, postnatal event complementing the functional impact of prenatal NOTCH1 mutation.

There are only 2 other studies addressing the possible prenatal origins of T-ALL. The first was our previous report of a pair of monozygotic twins with T-NHL/ALL.22 Malignant cells from the pair shared an identical TCRβ rearrangement (including an 11 bp N region) indicative of a single cell clonal origin in utero. Cells from these twins did not have a NOTCH1 mutation (A.M. Ford and M.G., unpublished observation, March 2007).

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Authorship

Contribution: M.E.-I. and M.E. carried out all experimental work. H.K. provided clinical samples and diagnostic details plus Guthrie cards. M.G. conceived and planned the study and wrote the paper.

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