Expression Levels of *TEL*, *AML1*, and the Fusion Products *TEL-AML1* and *AML1-TEL* versus Drug Sensitivity and Clinical Outcome in t(12;21)-Positive Pediatric Acute Lymphoblastic Leukemia

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Abstract

Purpose: t(12;21) (p13; q22), present in ~25% of pediatric precursor B-ALL, is highly sensitivity to L-asparaginase and the prognosis depends on the intensity of the treatment protocol. This study analyzes the relationship between the mRNA expression of the genes and fusion products involved in t(12;21), *in vitro* sensitivity to prednisolone, vincristine, and L-asparaginase, and long-term clinical outcome in t(12;21)+ acute lymphoblastic leukemia (ALL) patients.

Experimental Design: Long-term clinical outcome in 45 t(12;21)+ ALL patients was related to mRNA expression of *TEL*, *AML1*, *TEL-AML1*, and *AML1-TEL*, determined by real-time quantitative PCR, and the *in vitro* sensitivity to prednisolone, vincristine, and L-asparaginase, using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays.

Results: A significant ~3.5-fold lower *TEL* expression in t(12;21)+ compared with t(12;21)− ALL samples ($P = 0.006$) and normal controls ($P = 0.004$) was found. Expression of *AML1* did not differ between t(12;21)+ and t(12;21)− ALL. However, *AML1* expression in the leukemic cells was 2-fold higher compared with normal controls ($P = 0.02$). The *TEL-AML1* fusion product was expressed in all t(12;21)+ cases, whereas the reciprocal fusion product *AML1-TEL* was expressed in only 76%. High expression levels of *TEL-AML1* [hazard ratio (HR), 1.3; 95% confidence interval (95% CI), 1.10-1.57; $P = 0.003$], *AML1-TEL* (HR, 4.9; 95% CI, 1.99-12.40; $P = 0.001$) and *AML1* (HR, 1.1; 95% CI, 1.03-1.22; $P = 0.006$) were associated with a poor long-term clinical outcome within t(12;21)+ ALL. Cellular drug resistance towards prednisolone, vincristine, and L-asparaginase could not explain this predictive value. Multivariate analysis including age and WBC showed that only high *AML1-TEL* expression is an independent poor prognostic factor in t(12;21)+ childhood ALL.

Conclusion: High *AML1-TEL* expression is an independent poor prognostic factor in t(12;21)+ childhood ALL.

The t(12;21)(p13;q22) occurs in ~25% of childhood acute lymphoblastic leukemia (ALL) and is restricted to precursor B-cell leukemia. The t(12;21) involves fusion of the *TEL (ETV6)* gene at 12p13 with the *AML1 (CBFA2/RUNX1)* gene at 21q22. The breakpoint most often occurs in intron 5 of *TEL* and intron 1 of *AML1*. A frequent translocation variant results in fusion between intron 5 of *TEL* and intron 2 of *AML1*. The *TEL* gene is a member of the ETS family of transcription factors and functions as a transcriptional repressor (1). *AML1* encodes a transcription factor that acts as a transcription activator as well as a transcriptional repressor (2). Both genes are frequent targets of chromosomal translocations in a variety of myeloid and lymphoid leukemias (3, 4).

Since the discovery of t(12;21), several studies addressed the prognostic value of this particular translocation (reviewed by Loh and Lubnitz; ref. 5). In general, t(12;21)-positive ALL is associated with a favorable prognosis although conflicting results have been reported (5). In the Dutch Childhood Oncology Group ALL-7 and ALL-8 treatment protocols, no prognostic value was found for t(12;21)-positive ALL (6). In addition, the t(12;21)-positive ALL group does not seem to be a homogenous group, because ±20% of the Dutch t(12;21)-positive ALL patients relapsed.[5] Furthermore, additional genetic changes in *TEL* and *AML1* e.g., deletion of the nontranslocated *TEL* gene, an additional copy of *AML1*, an extra

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der(21)t(12;21), or combinations of these genetic abnormalities in t(12;21)-positive ALL are present in >80% of patients.[5] We recently showed that the absence of additional genetic changes in *TEL* and *AML1* as well as the presence of an extra der(21)t(12;21) are associated with an unfavorable prognosis within t(12;21)-positive ALL, which is not independent from prednisolone resistance.[5]

In the present study, we analyzed whether the expression levels of *TEL*, *AML1*, and the fusion products *TEL-AML1* and *AML1-TEL* are associated with drug sensitivity and long-term clinical outcome in t(12;21)-positive ALL.

Materials and Methods

Patient samples. Bone marrow and peripheral blood samples from untreated children with common/pre B-ALL at initial diagnosis were collected from the Erasmus MC, Sophia Children's Hospital, the Dutch Childhood Oncology Group and the German Childhood Acute Lymphoblastic Leukemia study group. Bone marrow and/or peripheral blood samples from the Erasmus MC, Sophia Children's Hospital from children who turned out to be nonleukemic were included as controls. Within 24 hours after sampling, mononuclear cells were isolated by density gradient centrifugation using Lymphoprep (density, 1.077 g/mL; Nycomed Pharma, Oslo, Norway). Contaminating nonleukemic cells in the ALL samples were removed by immunomagnetic beads as described earlier (7). All resulting samples contained ≥90% leukemic cells, as determined morphologically on May-Grünwald-Giemsa (Merck, Darmstadt, Germany) stained cytospins. For RNA extraction, a minimum of 5×10^6 leukemic cells were lysed in Trizol reagent (Life Technologies, Gaithersburg, MD) and stored at −80°C. Leukemic cells (25×10^3) were used for cytospin preparations and stored at −20°C.

Fluorescence* in situ *hybridization analysis. The presence of the t(12;21) was determined on cytospin preparations with dual-colored fluorescence *in situ* hybridization (FISH; ref. 8) using a digoxigenin-labeled cosmid from intron 1 to exon 2 of *TEL* (50F4), together with a biotinylated cosmid for the first five exons of AML1 (CO664). FISH probes were kind gifts of Dr. N. Sacchi, University of Milan, Italy (ref. 9; CO664) and Prof. Dr. P. Marynen, Human Genetics, University of Leuven, Belgium (ref. 10; 50F4). Probe 50F4 was visualized with Texas Red and probe CO664 with FITC. In t(12;21)-positive patients a yellow fusion spot will be visible denoting the der(21)t(12;21), one green signal for the normal *AML1* on chromosome 21, and one red signal for the normal *TEL* on chromosome 12 if not deleted. In all instances, two independent observers examined 100 to 300 interphase nuclei each.

In vitro *L-asparaginase, prednisolone, and vincristine cytotoxicity assay.* *In vitro* L-asparaginase, prednisolone, and vincristine cytotoxicity was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay as described previously (11). Briefly, 100-μL aliquots of cell suspension (1.6×10^5 cells) were cultured in round-bottomed 96-well microtiter plates in the presence of six concentrations of L-asparaginase (Paronal, Christiaens B.V., Breda, the Netherlands) ranging from 0.0032 to 10 IU/mL, prednisolone (Bufa B.V., Uitgeest, the Netherlands) ranging from 0.08 to 250 μg/mL, and vincristine sulfate (TEVA Pharma BV, Mijdrecht, the Netherlands) ranging from 0.049 to 50 μg/mL in duplicate. Control cells were cultured without L-asparaginase, prednisolone, or vincristine. After incubating the plates for 4 days at 37°C in humidified air containing 5% CO_2, 10 μL of 3-[4,5-dimethylthiazol-2-yl] 2,5-diphenyltetrazoliumbromide (5 mg/mL; Sigma Aldrich, Zwijndrecht, the Netherlands) were added. During a 6-hour incubation, the yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide tetrazolium salt is reduced to purple-blue formazan crystals by viable cells. Samples with ≥70% leukemic cells in the control wells and an absorbance higher than 0.050 arbitrary units (adjusted for blank values) were used to calculate the concentration of drug lethal to 50% of the cells (LC_{50}).

Real-time quantitative PCR. The t(12;21)-positive ALL patients with sufficient material available were selected to perform real-time quantitative PCR, but patients were selected without preexisting knowledge about the clinical outcome of these patients. Total cellular RNA was extracted from a minimum of 5×10^6 (≥90% leukemic) cells using Trizol reagent (Life Technologies) according to the manufacturer's protocol, with minor modifications that improved the quality of RNA. cDNA was synthesized using random hexamers and oligo dT as published previously (12). The mRNA expression levels of *TEL*, *AML1*, *TEL-AML1*, and *AML1-TEL* and as a reference the endogenous housekeeping gene encoding for glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), were quantified using real-time PCR analysis (Taqman chemistry) on an ABI Prism 7700 sequence detection system (PE Applied Biosystems, Foster City, CA). Amplification of specific PCR products was detected using dual-fluorescent nonextendable probes labeled with 6-carboxyfluorescein at the 5′-end and 6-carboxytetramethylrhodamine at the 3′-end. The primers and probe combinations were designed using the OLIGO 6.22 software (Molecular Biology Insights, Cascade, CO) and purchased from Eurogentec (Seraing, Belgium). The forward and reverse primer and probe combinations were, respectively, 5′-ACCCTCTGATCCTGAACC-3′, 5′-CCGTTGGGATCCACTATC-3′ and 5′-TCATCGGGAAGACCTGGCTT-3′ for *TEL*; 5′-GACAGCCCCAACTTCC-3′, 5′-CCACTTCGACCGACAA-3′ and 5′-CCTGCCCATCGCTTTCA-3′ for *AML1*; 5′-ACCCTCTGATCCTGAACC-3′, 5′-CATCCGTGGACGTCTCT-3′ and 5′-TCATCGGGAAGACCTGGCTT-3′ for *TEL-AML1*; 5′-GAGTCCCAGAGGTATCCAG-3′, 5′-AATCCCAAAGCAGTCTACA-3′ and 5′-TGACCTGTCTTGGTTTTCGC-3′ for *AML1-TEL*; 5′-GTCGGAGTCAACGGATT-3′, 5′-AAGCTTCCCGTTCTCAG-3′ and 5′-TCAACTACATGGTTTACATGTTCCAA-3′ for *GAPDH*. All primers had a melting temperature (T_m; nearest neighbor method) of 65°C to 66.5°C at salt concentration of 303 mmol/L of Na^+ equivalent and 300 nmol/L of primer concentration. Both internal probes had a T_m of 75 ± 1°C. All PCRs done at comparable efficiencies of ≥95%. The real-time quantitative PCR was done under the same conditions as described before (12). The comparative cycle time (C_t) value is the target PCR C_t value normalized by subtracting the *GAPDH* C_t value from the target PCR C_t value. From this ΔC_t value, the relative expression level to *GAPDH* in arbitrary units (AU) for each target PCR can be calculated using the following equation: relative mRNA expression = $2^{-\Delta Ct} \times 100$.

Statistics. Disease-free survival (pDFS) was calculated from the date of diagnosis to the date of nonresponse, relapse, or last contact. The failure to achieve complete remission at day 56 (nonresponse) was considered an event at day 56. pDFS curves were calculated according the Kaplan-Meier method and compared by the Cox proportional hazard regression model. Multivariate analysis was done with the Cox proportional hazard regression model. Statistical tests were done at a two-tailed significance level of 0.05. Differences in the distribution of variables between groups of patients were analyzed using the Mann-Whitney *U* test. Bivariate correlations were calculated using the Spearman's rank correlation test.

Results

The mRNA expression levels of *TEL*, *AML1*, *TEL-AML1*, and *AML1-TEL* were measured in 45 t(12;21)-positive pediatric ALL samples that were validated by FISH analysis. A control group of 26 t(12;21)-negative ALL samples was selected by matching for the following criteria: age 1 to 10 years, immunophenotype, no hyperdiploidy (>50 chromosomes), and no *MLL* rearrangements and no t(9;22). Furthermore, a nonleukemic control group containing both bone marrow and peripheral blood samples ($n = 14$) was selected to compare *TEL* and *AML1* expression in normal and leukemic cells.

TEL-AML1*, *AML1-TEL*, *AML1*, and *TEL* mRNA Expression and Drug Resistance. Expression of the fusion product *TEL-AML1*

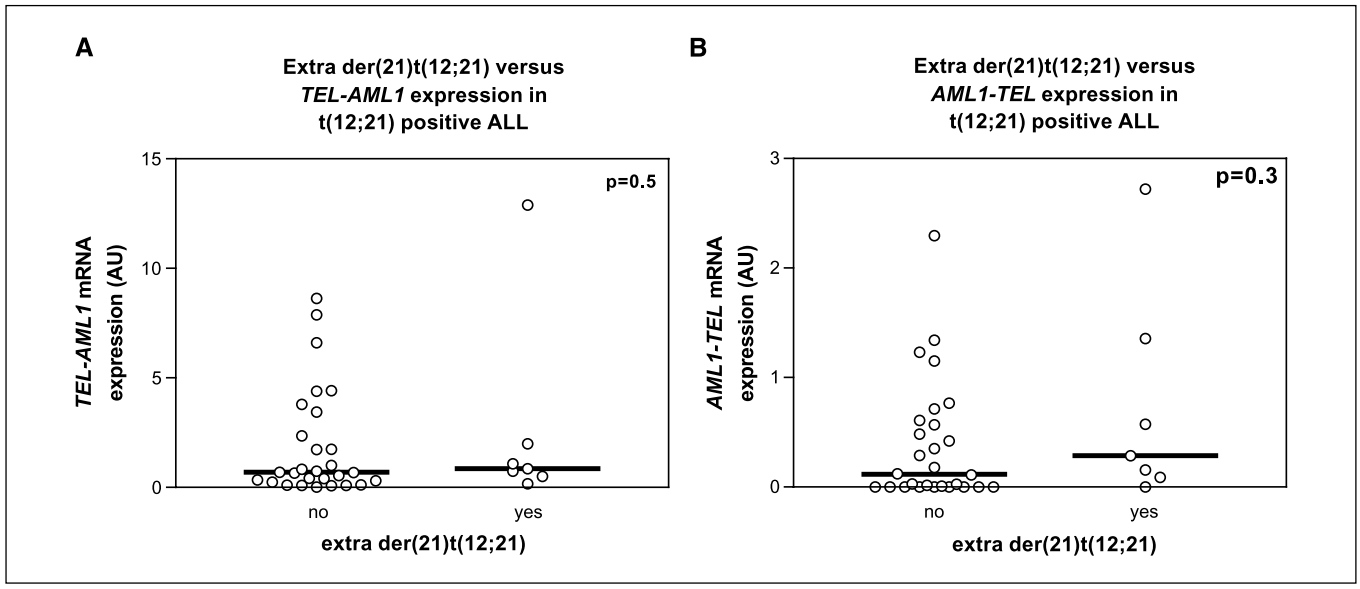

Fig. 1. Relationship between an extra copy of der(21)t(12;21) and expression of fusion products of t(12;21)-positive ALL. *A, TEL-AML1*. mRNA expression of *TEL-AML1* in t(12;21)-positive ALL patients with (n = 7; median, 0.85) and without (n = 28; median, 0.69) an extra der(21)t(12;21) (P = 0.5). *B, AML1-TEL*. mRNA expression of *AML1-TEL* relative to *GAPDH* in t(12;21)-positive ALL patients with (n = 7; median, 0.29) and without (n = 28; median 0.116) an extra der(21) t(12;21), P = 0.3.

was present in all 45 t(12;21)-positive ALL patients tested, whereas the *AML1-TEL* expression was present in only 76% of these cases. We compared the data on the presence of an extra der(21)t(12;21) to the mRNA expression of *TEL-AML1* and *AML1-TEL* (Fig. 1). The expression of these fusion genes did not differ between patients with and without an extra der(21)t(12;21) (P = 0.5 and P = 0.3, respectively). No significant correlation was found between the expression of these fusion genes and sensitivity to L-asparaginase, prednisolone, or vincristine ($-0.233 > R_s < 0.102$, $P > 0.05$).

Expression of *AML1* did not significantly differ between 45 t(12;21)-positive and 26 t(12;21)-negative ALL (P = 0.9; Fig. 2A). However, the mRNA expression of *AML1* in these 71 ALL samples (median, 5.15 AU) is 2-fold higher compared with 14 normal control bone marrow or peripheral blood samples (median, 2.30 AU; P = 0.02). Patients with an extra copy of *AML1* in t(12;21)-positive ALL do not have a higher expression of *AML1* (median, 1.3-fold difference; P = 0.4), as shown in Fig. 2B. No correlations were found between *AML1* mRNA expression and sensitivity to L-asparaginase, prednisolone, and vincristine within neither t(12;21)-positive ALL samples ($0.154 > R_s < 0.256$, $P > 0.05$) or t(12;21)-negative ALL samples ($-0.055 > R_s < 0.172$, $P > 0.05$).

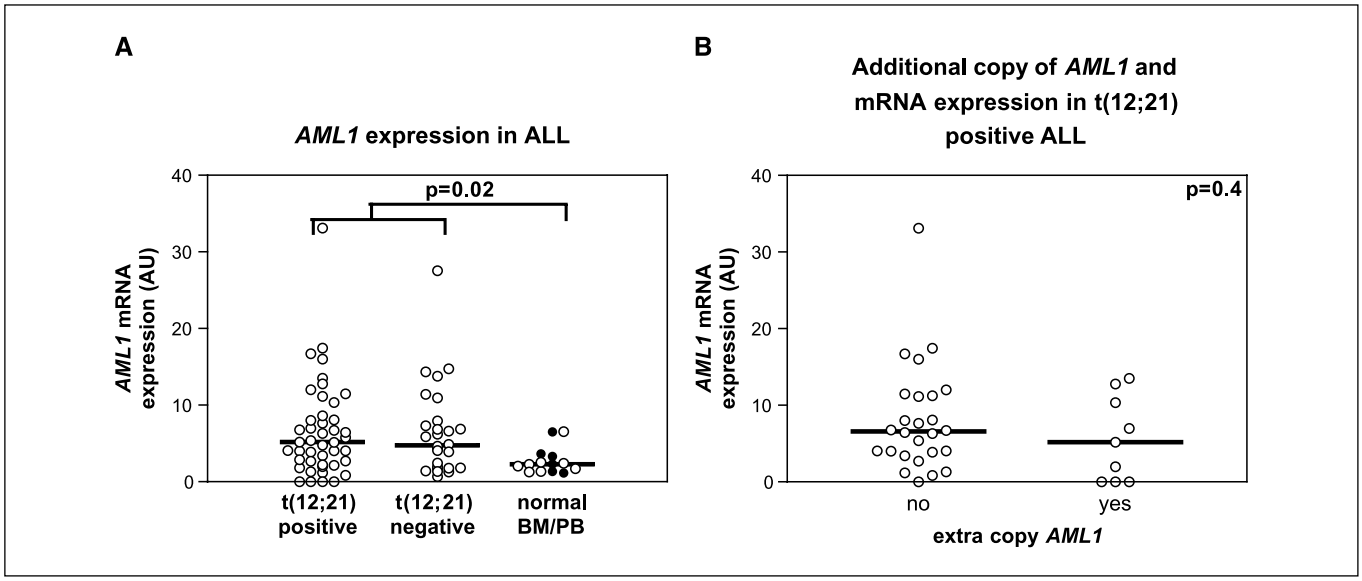

Fig. 2. *A, AML1* expression in ALL. mRNA expression of *AML1* in 45 t(12;21)-positive and 26 t(12;21)-negative ALL patients as well as 14 normal controls. Lines, median value; ○, bone marrow (*BM*); ●, peripheral blood (*PB*). t(12;21)-positive versus t(12;21)-negative patients (median, 5.19 and 4.75, respectively; P = 0.9); all ALL patients versus normal controls (median, 5.15 and 2.30, respectively; P = 0.02). *B,* extra copy of *AML1* versus *AML1* expression. mRNA expression of *AML1* in t(12;21)-positive ALL patients with (n = 9; median, 5.19) and without an extra copy of *AML1* (n = 26; median, 6.58; P = 0.4).

A significant 3.5-fold lower expression of *TEL* mRNA was observed in 33 t(12;21)-positive ALL (median, 0.10 AU) compared with 23 t(12;21)-negative ALL (median, 0.33 AU; $P = 0.006$) and 13 normal bone marrow or peripheral blood samples (median, 0.39 AU; $P = 0.004$; Fig. 3A). No significant difference in *TEL* mRNA expression was observed between 23 t(12;21)-negative ALL and 13 normal bone marrow or peripheral blood samples ($P = 0.3$). FISH analysis of t(12;21)-positive ALL samples indicated that in 70% of the cases a deletion of the nontranslocated *TEL* allele had occurred. Interestingly, patients with a deletion of the nontranslocated *TEL* allele still shown detectable *TEL* mRNA expression levels (Fig. 3B). Further examination revealed that this mRNA is derived from contaminating normal cells in the t(12;21)-positive ALL samples (Fig. 3C). Despite the fact that our samples were purified towards >90% leukemic cells, the presence of <10% contaminating normal cells contributed to detectable *TEL* mRNA levels. Furthermore, FISH analysis showed that a deletion of the second *TEL* allele is not present in 100% of the leukemic cells. Besides the dominant t(12;21)-positive clone, smaller t(12;21)-positive clones were observed that had retained the nontranslocated *TEL* allele. Therefore, expression of *TEL* in t(12;21)-positive patients with a deletion of the nontranslocated *TEL* allele in the dominant t(12;21)-positive clone is probably the result of *TEL* expression in contaminating nonleukemic cells and small t(12;21)-positive subclones which retained the *TEL* allele. No correlations were found between *TEL* expression and sensitivity to L-asparaginase, prednisolone, and vincristine within both t(12;21)-positive ALL ($-0.105 > R_s < 0.379$, $P > 0.05$) and t(12;21)-negative ALL ($-0.041 > R_s < 0.362$, $P > 0.05$).

TEL-AML1, AML1-TEL, and AML1 mRNA Expression and Clinical Outcome. Patients with a high expression of *TEL-AML1* (mRNA expression above 75th percentile of total group) had a poorer outcome (3-year pDFS 30 ± 25%) than those with low expression levels of *TEL-AML1* (mRNA

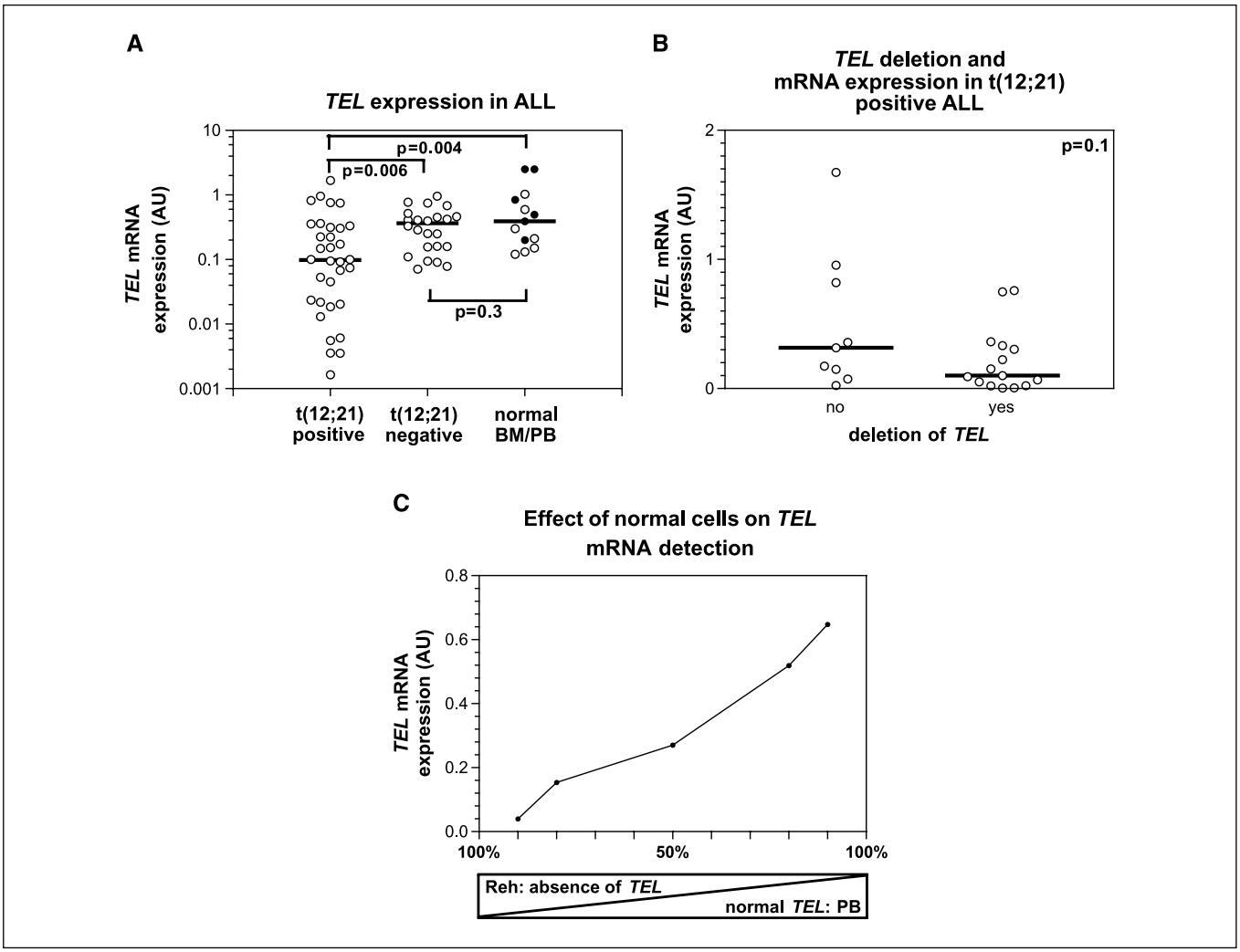

Fig. 3. *A, TEL* expression in ALL. mRNA expression of *TEL* in 33 t(12;21)-positive and 23 t(12;21)-negative ALL patients as well as in 13 normal controls. Lines, median value; ○, bone marrow (*BM*); ●, peripheral blood (*PB*). t(12;21)-positive versus t(12;21)-negative patients (median, 0.10 and 0.33, respectively; $P = 0.006$); t(12;21)-positive versus normal controls (median, 0.10 and 0.39, respectively; $P = 0.004$); t(12;21)-negative patients versus normal controls (median, 0.33 and 0.39, respectively; $P = 0.3$). *B,* deletion of *TEL* versus *TEL* expression. mRNA expression of *TEL* in t(12;21)-positive ALL patients with ($n = 15$; median, 0.10) or without ($n = 9$; median, 0.32) a deletion of the nontranslocated *TEL* allele ($P = 0.1$). *C,* effect of normal cells on *TEL* mRNA detection. A dilution series of 0% *TEL* expression (Reh cell line: 100% t(12;21)-positive blasts with a deletion of the nontranslocated *TEL* allele) to 100% *TEL* expression in normal peripheral blood (*PB*). Already low amounts (10%) of normal peripheral blood cells result into detectable *TEL* expression levels, comparable to the median expression observed in the t(12;21)-positive ALL group with a deletion of *TEL* (*B*).

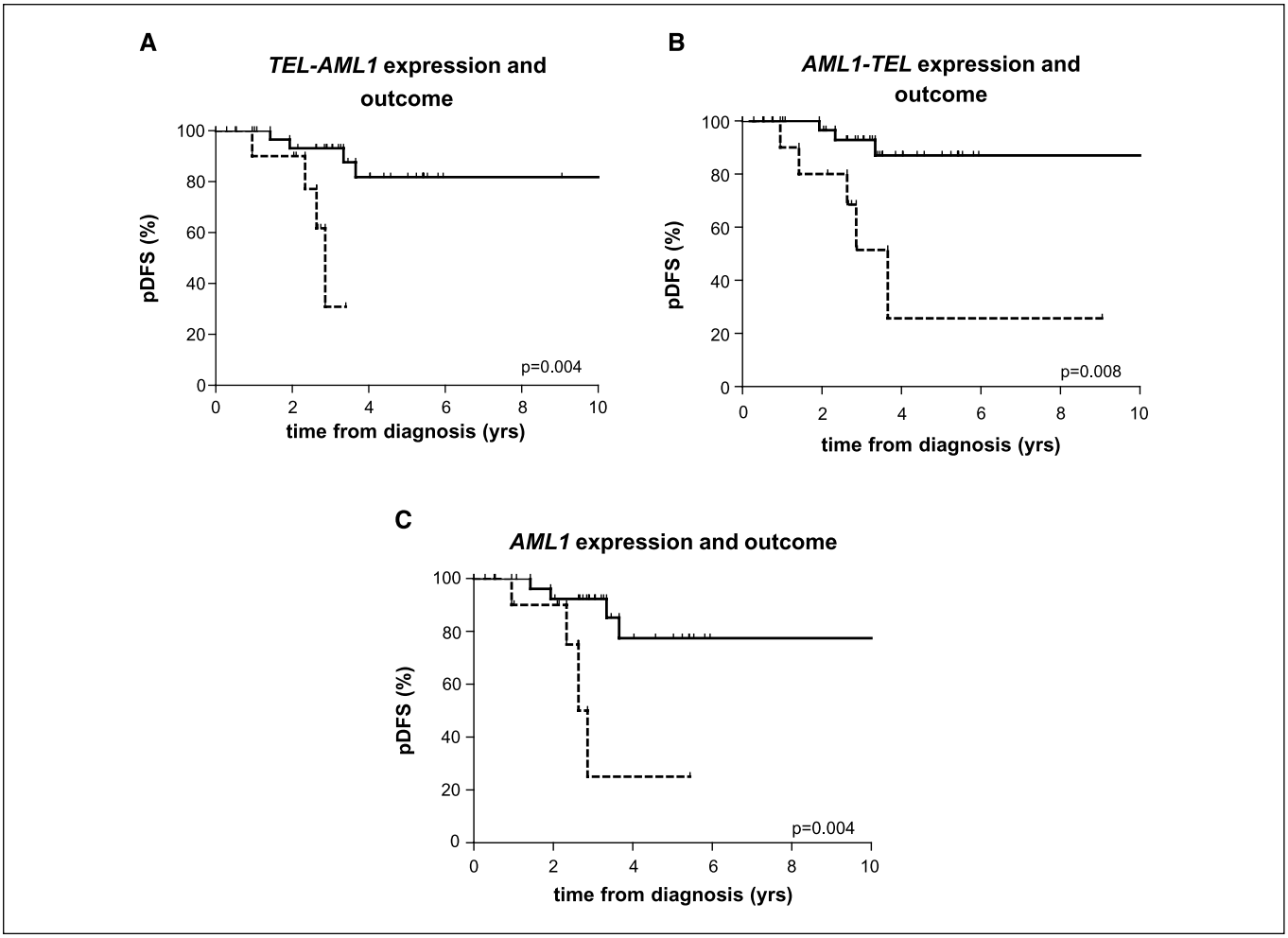

Fig. 4. Patients were divided into two groups by the 75th percentile for the expression of *TEL-AML1* (*A*), *AML1-TEL* (*B*), and *AML1* (*C*). Dashed line, high expression (P > 75); solid line, low expression (P < 75). Differences in pDFS were analyzed by the Cox proportional hazard regression model (P_{cox}). *A,* high expression is associated with an unfavorable prognosis (3-year pDFS 30 ± 25%) compared with low expression (3-year pDFS 93 ± 5%; P_{cox} = 0.004; $P_{cox\ continous\ variable}$ = 0.003; see Table 2). *B,* high expression is associated with an unfavorable prognosis (3-year pDFS 51 ± 25%) compared with low expression (3-year pDFS 93% ± 6%; P_{cox} = 0.008; $P_{cox\ continous\ variable}$ = 0.001; see Table 2). *C,* high expression is associated with an unfavorable prognosis (3-year pDFS 25 ± 22%) compared with low expression (3-year pDFS 93 ± 5%; P_{cox} = 0.004; $P_{cox\ continous\ variable}$ = 0.006; see Table 2).

expression below 75th percentile of total group; 3-year pDFS 93 ± 5%; P = 0.004; Fig. 4A). In addition, high expression of *AML1-TEL* (3-year pDFS 51 ± 25%; P = 0.008; Fig. 4B) and *AML1* (3-year pDFS 25 ± 22%; P = 0.004; Fig. 4C) were related to a poor outcome. Cox regression analysis using the mRNA expression levels of *TEL-AML1*, *AML1-TEL*, and *AML1* as continuous variables also indicated that an increase in expression is associated with an increase in relapse risk in t(12;21)-positive ALL, whereas an increase in *AML1* expression in t(12;21)-negative ALL does not relate with an increased relapse risk (Table 1). In addition, a multivariate analysis including also the known prognostic factors age and WBC at diagnosis was done (Table 2). In this analysis, only increased expression of *AML1-TEL* was associated with a poor prognosis in t(12;21)-positive ALL (hazard ratio, 7.02; 95% confidence interval, 2.01-24.52; P = 0.002). High expression of *TEL-AML1* mRNA was correlated with high expression of *AML1* (R_s = 0.524; P < 0.001). This can explain the fact that both *TEL-AML1* and *AML1* are no independent prognostic factors in the multivariate analysis. The expression of *AML1-TEL* was not related to either *TEL-AML1* or *AML1* expression (R_s = 0.126, P = 0.4 and R_s = 0.097, P = 0.5, respectively).

Discussion

In the present study, we examined the relation among *TEL*, *AML1*, *TEL-AML1*, and *AML1-TEL* mRNA expression, the additional genetic changes in *TEL* and *AML1* genes, the *in vitro* sensitivity to L-asparaginase, prednisolone, and vincristine, and clinical outcome in children with t(12;21)-positive ALL at initial diagnosis.

A significantly lower expression of *TEL* was found in t(12;21)-positive ALL patients compared with t(12;21)-negative ALL patients and normal controls. This can be explained by the fact that the nontranslocated *TEL* allele is frequently (±70%) deleted in t(12;21)-positive ALL[5] (13–15). Thus far, only one earlier study analyzed the mRNA expression of wild-type *TEL* in childhood acute leukemia (16). In contrast to our results, Patel et al. found no difference in *TEL* expression between t(12;21)-positive ALL and a control group, but these authors did not

Table 1. Predictive value of fusion gene and *AML1* expression in t(12;21)-positive and negative ALL

Variable	*n*	Hazard ratio*	95% confidence interval	*P*
t(12;21)-positive ALL patients				
TEL-AML1 expression	45	1.32	1.10-1.57	0.003
AML1-TEL expression	45	4.97	1.99-12.40	0.001
AML1 expression	45	1.12	1.03-1.22	0.006
t(12;21)-negative ALL patients				
AML1 expression	26	0.87	0.74-1.03	0.1

*Univariate Cox proportional hazard analysis using mRNA expression levels as continuous variable.

specify the percentage of blasts in their t(12;21)-positive ALL samples. In the present study, we observed that low expression levels of *TEL* may originate from contaminating nonleukemic cells present in t(12;21)-positive ALL samples with a deletion of the second *TEL* allele and from small t(12;21)-positive subclones retaining the *TEL* allele.

No difference in *AML1* expression was found between t(12;21)-positive ALL patients (with or without an additional copy of *AML1*) and t(12;21)-negative ALL patients, although only in t(12;21)-positive ALL patients high *AML1* expression is related to a poor outcome. Our data show that *AML1* expression is significantly elevated in both ALL subgroups compared with the normal control group. This might be due to the fact that expression of *AML1* is required for proliferation since *AML1* regulates G_1 to S cell cycle transition (17, 18). Although the AML1 expression in t(12;21)-positive and t(12;21)-negative ALL is comparable, differences in cell cycle are present in these two groups of ALL. The percentage of cells in S phase is lower in t(12;21)-positive ALL compared with t(12;21)-negative ALL (19). This might be explained by the fact that in t(12;21)-positive patients TEL-AML1 competes with wild-type AML1 to bind the DNA and acts as a transcriptional repressor of AML1 target genes (20).

Conflicting data on the prognostic relevance of t(12;21)-positive ALL have been reported ranging from 60% to 100% survival (reviewed by Loh et al.; refs. 5, 21–24). Initially, studies reported favorable outcome of t(12;21)-positive ALL patients compared with t(12;21)-negative ALL patients. Later on, several studies could not confirm this prognostic relevance, among which Dutch Childhood Oncology Group–treated t(12;21)-positive ALL[5] (6). The intensity of treatment given to t(12;21)-positive ALL patients seems to contribute to a favorable outcome (25–27). Our data show that a high expression of *TEL-AML1*, *AML1-TEL*, and *AML1* are related to a poor prognosis in pediatric t(12;21)-positive ALL. However, only the expression of *AML1-TEL* is an independent prognostic factor in t(12;21)-positive pediatric ALL. Current research focuses on determining the function of the TEL-AML1 fusion protein in leukemogenesis, because the *TEL-AML1* fusion product is expressed in all t(12;21)-positive ALL cases, whereas the reciprocal fusion product *AML1-TEL* is not. In mouse models, TEL-AML1 alone is insufficient for leukemogenesis but may result in leukemia when additional mutations are present (28, 29). The presence of AML1-TEL expression did not make a difference in inducing hematologic disease in transgenic mice (28). The *TEL-AML1* fusion product was detected in neonatal blood spots and cord blood samples at a hundred times higher frequency than expected from the corresponding leukemia incidence (30, 31). This finding together with the mouse model studies suggests that secondary additional genetic changes are required for leukemogenesis. As the second *TEL* allele is most frequently deleted in t(12;21)-positive ALL, it is speculated that wild-type *TEL* acts as a tumor suppressor gene and its deletion in t(12;21)-positive ALL is the second hit required for leukemogenesis. However, 30% of the t(12;21)-positive ALL patients do not show a deletion of *TEL* indicating that another genetic abnormality is necessary for leukemogenesis. In a previous study, we showed the absence of additional genetic changes in *TEL* and *AML1* genes as well as an extra der(21)t(12;21) are associated with an unfavorable prognosis in pediatric t(12;21)-positive ALL.[5] As shown in the present study, expression levels of *TEL-AML1* and *AML1* were not increased in patients with an extra der(21)t(12;21) or an additional copy of *AML1* respectively. However, this might be due to the fact that the discrimination level of the RTQ-PCR is minimal 2-fold.

As shown in the present study, *AML1-TEL* expression levels are associated with outcome. Resistance to prednisolone, vincristine, or L-asparaginase cannot explain this predictive value. Therefore, *AML1-TEL* may be involved in cell regrowth rather than in toxic response pathways. The *AML1-TEL* fusion product contains exon 1 or exons 1 and 2 of *AML1*, in which no functional domain is present and the last three exons of *TEL*, which contains the ETS domain. In the *TEL* gene, a repression domain was identified which encompasses the ETS

Table 2. Multivariate analysis of risk factors in t(12;21)-positive ALL

Variable	Unfavorable feature	Hazard ratio*	95% confidence interval	*P*
Age at diagnosis (y)	>10	9.05	0.47-173.31	0.14
WBC at diagnosis (10^9 cells/L)	>25	4.70	0.53-41.90	0.17
TEL-AML1 expression		1.23	0.79-1.93	0.36
AML1-TEL expression		7.02	2.01-24.52	0.002
AML1 expression		1.10	0.84-1.43	0.49

*Cox proportional hazard analysis using mRNA expression levels as continuous variable.

domain and the 50 amino acids immediately upstream of the ETS domain (1). It is hypothesized that ETS proteins with transcriptional repression activity (like TEL) are primarily involved in ensuring the balance between cellular proliferation and differentiation in different cell types and developmental stages, in response to extracellular signals (32). The isolated ETS domain of TEL binds conventional ETS binding sites *in vitro* and regulates ETS binding site–driven transcription (33–35). It can be hypothesized that AML1-TEL acts comparable to an isolated ETS domain and competes for binding with the endogenous TEL or acts like TEL in the absence of wild-type TEL. Therefore, it seems unlikely that AML1-TEL will not have a function in t(12;21)-positive ALL. This is the first study showing that *AML1-TEL* expression is associated with prognosis in t(12;21)-positive ALL. Further characterization and validation of AML1-TEL expression is required to determine the therapeutic implications of the *AML1-TEL* expression levels in t(12;21)-positive ALL.

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