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RESEARCH ARTICLE

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MYB rearrangements and over-expression in T-cell acute lymphoblastic leukemia

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Abstract

We investigated *MYB* rearrangements (*MYB*-R) and the levels of *MYB* expression, in 331 pediatric and adult patients with T-cell acute lymphoblastic leukemia (T-ALL). *MYB*-R were detected in 17 cases and consisted of *MYB* tandem duplication (tdup) (= 14) or T cell receptor beta locus *(TRB)-MYB* (= 3). As previously reported, *TRB-MYB* was found only in children (1.6%) while *MYB* tdup occurred in both age groups, although it was slightly more frequent in children (5.2% vs 2.8%). Shared features of *MYB*-R T-ALL were a non-early T-cell precursor (ETP) phenotype, a high incidence of *NOTCH1/FBXW7* mutations (81%) and *CDKN2AB* deletions (70.5%). Moreover, they mainly belonged to *HOXA* (=8), *NKX2-1/2-2/TLX1* (=4), and *TLX3* (=3) homeobox-related subgroups. Overall, *MYB*-R cases had significantly higher levels of *MYB* expression than *MYB* wild type (*MYB*-wt) cases, although high levels of *MYB* were detected in ~ 30% of *MYB*-wt T-ALL. Consistent with the transcriptional regulatory networks, cases with high *MYB* expression were significantly enriched within the *TAL/LMO* subgroup (P = .017). Interestingly, analysis of paired diagnosis/remission samples demonstrated that a high *MYB* expression was restricted to the leukemic clone. Our study has indicated that

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different mechanisms underlie *MYB* deregulation in 30%-40% of T-ALL and highlighted that, *MYB* has potential as predictive/prognostic marker and/or target for tailored therapy.

KEYWORDS
MYB expression, *MYB* tandem duplication, T-ALL, T-cell acute lymphoblastic leukemia, *TRB-MYB*

1 | INTRODUCTION

MYB has been identified as the cellular counterpart of the transforming v-Myb gene of the avian myeloblastosis virus and of the avian leukemia virus E26.[1] It encodes for a 75 kDa nuclear transcription factor, mostly operating as a transcriptional activator, that governs proliferation, differentiation, cell cycle, apoptosis, cell signaling, angiogenesis, and cell adhesion.[1] The protein consists of three major domains: A N-terminal DNA-binding domain that recognizes a consensus PyAACG/TG sequence motif involved in protein-protein interactions, a central transactivation domain, required to activate *MYB* targets, and a C-terminal negative autoregulatory domain, mediating post-translational modification.[1,2] The transcriptional activity of *MYB* is regulated by several coregulators that are necessary to mediate the activation of MYB targets and the interaction with other proteins.[1] *MYB* is predominantly expressed in colon crypts, breast epithelial cells and the hematopoietic compartment.[1] In the latter, it is essential for proliferation, lineage commitment, and differentiation of hematopoietic stem cells and progenitors[1]; however, studies in conditional knockout mice have also proved that *MYB* is required for normal T and B cells development.[3,4]

The oncogenic activity of *MYB* is mainly exerted in tissues, where it plays a pivotal role in development and maintenance. In fact, elevated MYB expression is more common in colon, breast cancer and hematological diseases.[1,2,5]

In hematological malignancies, aberrant *MYB* expression has been reported in acute myeloid leukemias, especially cases with a normal karyotype,[6] and a subset of T-cell acute lymphoblastic leukemia/lymphoma (T-ALL/LBL), either harbouring a t(6;7)(q23;q34) translocation, juxtapositing the T cell receptor beta locus *(TRB)* enhancer to *MYB*, or a genomic tandem duplication of the *MYB* locus on the long arm of chromosome 6.[7,8] Although reported in a single case of T-ALL, extrachromosomal amplification of *MYB*, appears to be an additional mechanism leading to *MYB* over-expression, and a recurrent somatic mutation, which is assumed to perturb the cellular localization and activity of *MYB*, has been recently detected in 2.8% of paediatric T-ALL.[9,10] Moreover, *MYB* oncogenic fusions promoting its transcriptional activity, have been reported in acute basophilic leukemia, with the t(X;6)(p11;q23)/*MYB-GATA1* translocation,[11] and in blastic plasmacytoid dendritic cell neoplasm, where *MYB* rearranges with *PLEKHO1*, *ZFAT*, *DCPS*, or *MIR3134*.[12] When ectopically transcribed, *MYB* blocks differentiation and promotes proliferation.[2,5]

Due to its oncogenic role in multiple cancers, *MYB* has been regarded as a potential therapeutic target. Different strategies, such as direct silencing, disruption of the MYB-p300 axis and/or the administration of BET inhibitors, have provided encouraging results in in vitro and in vivo preclinical studies.[1] Moreover, although results are not yet available, a phase I/II clinical trial with *MYB* antisense oligonucleotide is underway in advanced hematological malignancies (NCT00780052).

2 | MATERIALS AND METHODS

2.1 | Patients

The study was carried out on a cohort of 331 patients with T-ALL, previously reported.[13] There were 191 children and 140 adults (Table 1). Molecular-cytogenetics detected "type A" abnormalities in 237 cases which were classified as *TAL/LMO* (= 72), *HOXA* (*n* = 80), *TLX3* (*n* = 35), *TLX1* (*n* = 32), *NKX2-1/NKX2-2* (*n* = 15), and *MEF2C* (*n* = 3).[14] Ninety-four T-ALL remained undetermined[13] *CDKN2AB* mono- or bi- allelic deletions were detected 195/331 (59%) cases. *NOTCH1/FBXW7* hot spot mutations were found in 130/205 T-ALL cases for which DNA was available (63%). Patients or their parents/guardians gave informed consent for sample collection and molecular analyses, in agreement with the Declaration of Helsinki. The study was approved by the local bio-ethical committee (research project 3397/18).

2.2 | Molecular and cytogenetic studies

MYB rearrangements (*MYB*-R) were investigated with two specific fluorescence in situ hybridization (FISH) probe sets to detect either *MYB* tandem duplication (*MYB* tdup) or *TRB-MYB* (Figure 1). To confirm the *TRB-MYB* rearrangement, double color double fusion FISH assays with genomic clones for *TRB* (RP11-1220K2/RP11-556I13, labeled in green) and *MYB* (RP1-32B1/RP11-141K5, labeled in orange) were performed. Probes for the centromeric region of chromosome 6 and for genes/loci mapping to 6q15-q21 were selected to fully characterize cases in which *MYB* FISH probe showed abnormal hybridization patterns. The analysis was carried out on 100-150 nuclei using a fluorescence microscope (Olympus BX61).[13]

Single nucleotide polymorphism array (SNPa) was done on 102 cases (41 children and 61 adults) to validate *MYB* copy number

variations (CNV).[15] As the *MYB* tdup has variable sizes, we carried out an analysis using 100 Kb markers or no filter setting.[10]

Sanger sequencing was performed on 84 patients (57 adults and 27 children; 59 males and 25 females) to investigate *MYB* exon 2 hot spot mutations at codon 14,[10] using the following primers: (Fw 5'-GGAATAGGAAGGTGCCAGGT-3′, Rev 5'-CACATGCGGGCTAGGATAAG-3′). *MYB* exon 2 refers to accession number [NM_001130173.2].

TABLE 1 T-ALL cases investigated by fluorescence in situ hybridization for *MYB* rearrangements

	Children 191	Adults 141	Cohort 331
Age range	1-18	19-78	1-78
Sex			
Males	140	100	240
Females	51	40	91
Immunophenotype			
ETP/near- ETP	17	42	59
no-ETP	107	80	187
Not available	67	18	85
Classification			
TAL/LMO	57	15	72
HOXA	38	42	80
TLX3	26	9	35
TLX1	9	23	32
NKX2-1/2–2	14	1	15
MEF2C	1	2	3
Unclassified	39	46	85
Not available	8	1	9
Additional Abnormalities			
CDKN2AB deletion	125	70	195
NOTCH1/FBXW7 mutation	67	63	130

Abbreviation: T-ALL, T-cell acute lymphoblastic leukemia.

2.3 | Quantitative reverse transcription polymerase chain reaction (RT-qPCR)

MYB expression was investigated by RT-qPCR, in 76/331 patients (56 adults and 20 children) (Table 2). They included: 24 *HOXA*, 14 *TAL/LMO*, 17 *TLX1/3*, 1 *MEF2C*, and 20 undetermined cases; 7 with *MYB* rearrangements (2 *TRB-MYB* and 5 with *MYB* tdup) and 69 without, that is, *MYB* wild type (*MYB*-wt). In seven cases with available material (one *TRB-MYB*, one *MYB* tdup, and five *MYB*-wt), paired diagnostic/remission samples were investigated.

Total RNA was isolated using Trizol (Invitrogen, Thermo Scientific) or the QIAamp RNA blood MiniKit (Qiagen) from bone marrow or peripheral blood cells. The SuperScript IV First-Strand Synthesis System (Invitrogen) and esa-random primers (Invitrogen) were used to synthesize cDNA. *MYB* expression was investigated using TaqMan methods (TaqMan assay probe Hs00920556_m1; Applied Biosystems). All samples were analyzed in triplicate using Light Cycler 480 (LC480, Roche) and the gene expression was normalized to the endogenous reference controls *ABL1* (Hs00245445_m1; Applied Biosystems) and GUSB (Hs00939627_m1; Applied Biosystems). Universal Human Reference RNA (Stratagene, La Jolla, California) was used as calibrator in all experiments. Fluorescence data were analyzed with the software version 1.5 and second derivative maximum method. GraphPad Prism 5.0 was used for statistical analyses. Intergroup differences were analyzed by non-\parametric tests. The Mann-Whitney *U* test was used to compare differences between groups (*P* values <.05).

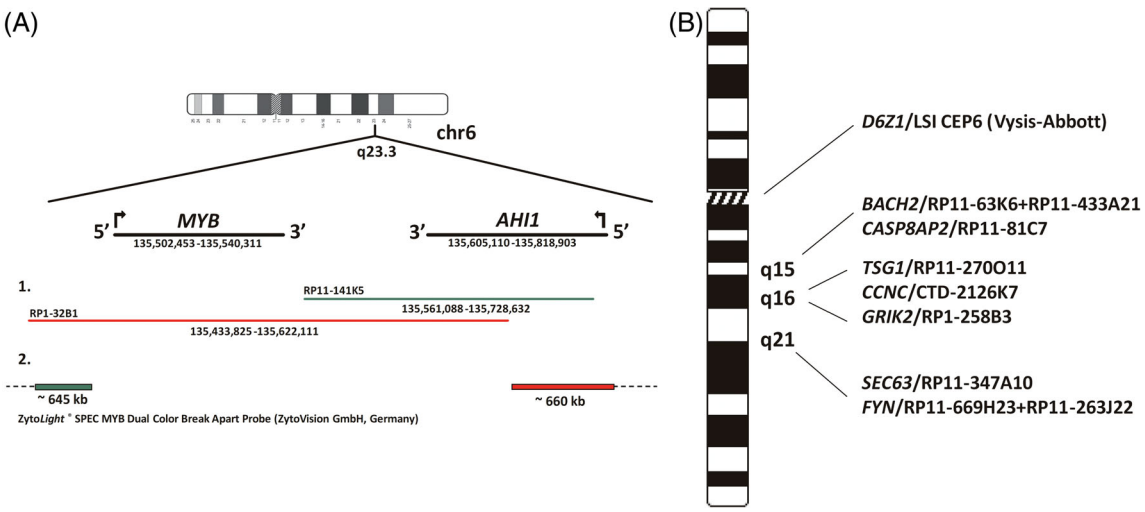

FIGURE 1 *MYB* probes used for FISH experiments. A, Schematic representation of the two fluorescence in situ hybridization (FISH) assays used to study *MYB*: RP1-32B1 labelled with SpectrumOrange and RP11-141K5 labelled with SpectrumGreen (1) and ZytoVision *MYB* break apart probe (2). Genomic position refers to GRCh37/hg19 assembly. Image are not to scale. B, The schematic ideogram of chromosome 6 shows the mapping of genomic clones used to investigate genes that map at 6q15-6q21 bands

TABLE 2 Characteristics of 76 T-ALL patients investigated by qRT–PCR

	Children 20	Adults 56	Cohort 76
Age range	1-18	19-78	1-78
Sex			
Males	12	39	51
Females	8	17	25
Classification			
TAL/LMO	5	9	14
HOXA	7	17	24
TLX3	3	5	8
TLX1	0	9	9
MEF2C	1	0	1
Unclassified	4	16	20
MYB Abnormalities			
MYB-R	3	4	7
MYB-wt	17	52	69
Additional Abnormalities			
CDKN2AB deletion	11	31	42
NOTCH1/FBXW7 mutation	15	32	47

Abbreviations: *MYB*-R, *MYB* rearrangements; *MYB*-wt, wild type; qRT–PCR, quantitative reverse transcription polymerase chain reaction; T-ALL, T-cell acute lymphoblastic leukemia.

3 | RESULTS

3.1 | Molecular-cytogenetic studies

Integrated molecular-cytogenetic studies detected *MYB*-R in 17/331 T-ALL (Table 1 and 3). There were three *TRB-MYB* and 14 *MYB* tandem duplication (tdup) (Table 3 and Figure 2A,B,C). FISH detected three balanced *TRB-MYB* rearrangements (Figure 2A,B) and 13 *MYB* tdup (Figure 2C). SNPa confirmed all *MYB* tdup and identified an additional case (no. 10 of Table 3, Figure 2D) in which the duplicated region was 100 Kb in size. SNPa also informed on the number of *MYB* copies involved in the tandem duplication, which were two (six cases), three (two cases), or four (one case) (Figure 2E). In the latter case, FISH confirmed a local rearrangement, in which all of the extra copies of *MYB* were closely apposed. Other *MYB* CNV, detected in our cohort, consisted of large 6q duplication (six cases) or trisomy 6 (eight cases) (data not shown). No mutation at *MYB* codon 14 was detected in the 84 T-ALL cases investigated.

3.2 | Quantitative reverse transcription polymerase chain reaction

Overall, patients had a wide range of *MYB* relative expression (Figure 3A) which was, however, significantly higher in *MYB*-R than *MYB*-wt cases (by 1.8-fold) (Mann-Whitney *U* test, $P = .003$) (Figure 3B). Among

TABLE 3 Clinical, hematological, and molecular-cytogenetic features of 17 patients with *MYB* rearrangements

Patients	S/A	*MYB*-R	CI-FISH	*NOTCH1*, *FBXW7*	Genetic Group
1	M/1	*TRB-MYB*	*CDKN2AB* del	MUT	UNCLASSIFIED
2	M/5	*TRB-MYB*	*BCL11B-HOXA*, *CDKN2AB* del	MUT	*HOXA*
3	F/10	*TRB-MYB*	*BCL11B-TLX3*, *CDKN2AB* del, trisomy 8, trisomy 18	MUT	*TLX3*
4	M/4	*MYB* tdup	*BCL11B-TLX3*, *CDKN2AB* del, *ETV6-CDKN1B* del	MUT	*TLX3*
5	F/39	*MYB* tdup	*KMT2A*-translocation, *TCF7* del, del(6q)/ *CASP8AP2-GRIK2-SEC63-FYN*, *PTEN* del	WT	*HOXA*
6	M/29	*MYB* tdup	*TLX1*-translocation, dup(9q)/*ABL1-NUP214-NOTCH1*, *CDKN2AB delPTPN2* del, *RB1 del*	MUT	*TLX1*
7	M/25	*MYB* tdup	*NUP98-translocation*, *TCF7 del*	MUT	*HOXA*
8	M/31	*MYB* tdup	*DDX3X-MLLT10*	MUT	*HOXA*
9	F/13	*MYB* tdup	*CALM-MLLT10*, *CDKN2AB* del, *LEF1* del, del*(1)(p32)*	MUT	*HOXA*
10	M/3	normal	*CDKN2A/B* del, *CALM-MLLT10*, *BCL11B* del	MUT	*HOXA*
11	F/16	*MYB* tdup	*NUP98-RAP1GDS1*, trisomy 8	MUT	*HOXA*
12	M/6	*MYB* tdup	*TLX1*-translocation, *CDKN2AB* del, *PTPN2* del	N.A	*TLX1*
13	M/12	*MYB* tdup	*CALM-MLLT10*, *CDKN2AB* del	N.A	*HOXA*
14	F/8	*MYB* tdup	*BCL11B-NKX2-1*, *TRAD* del	WT	*NKX2-1/2-2*
15	M/5	*MYB* tdup	*CDKN2AB* del, del(6q)/*CASP8AP2*, *TCRB* del, dup(9q)/ *TAL2-ABL1-NUP214*	N.A	UNCLASSIFIED
16	M/14	*MYB* tdup	*BCL11B-TLX3*, *CDKN2AB* del	WT	*TLX3*
17	M/4	*MYB* tdup	*TRAD-NKX2-1*, *CDKN2AB* del, *PTPN2* del	MUT	*NKX2-1/2-2*

Abbreviations: A, age; CI-FISH, combined interphase fluorescence in situ hybridization; F, female; M, male; MUT, mutated; *MYB*-R, *MYB* rearrangements; N.A., not available; S, sex; tdup, tandem duplication; WT, wild type.

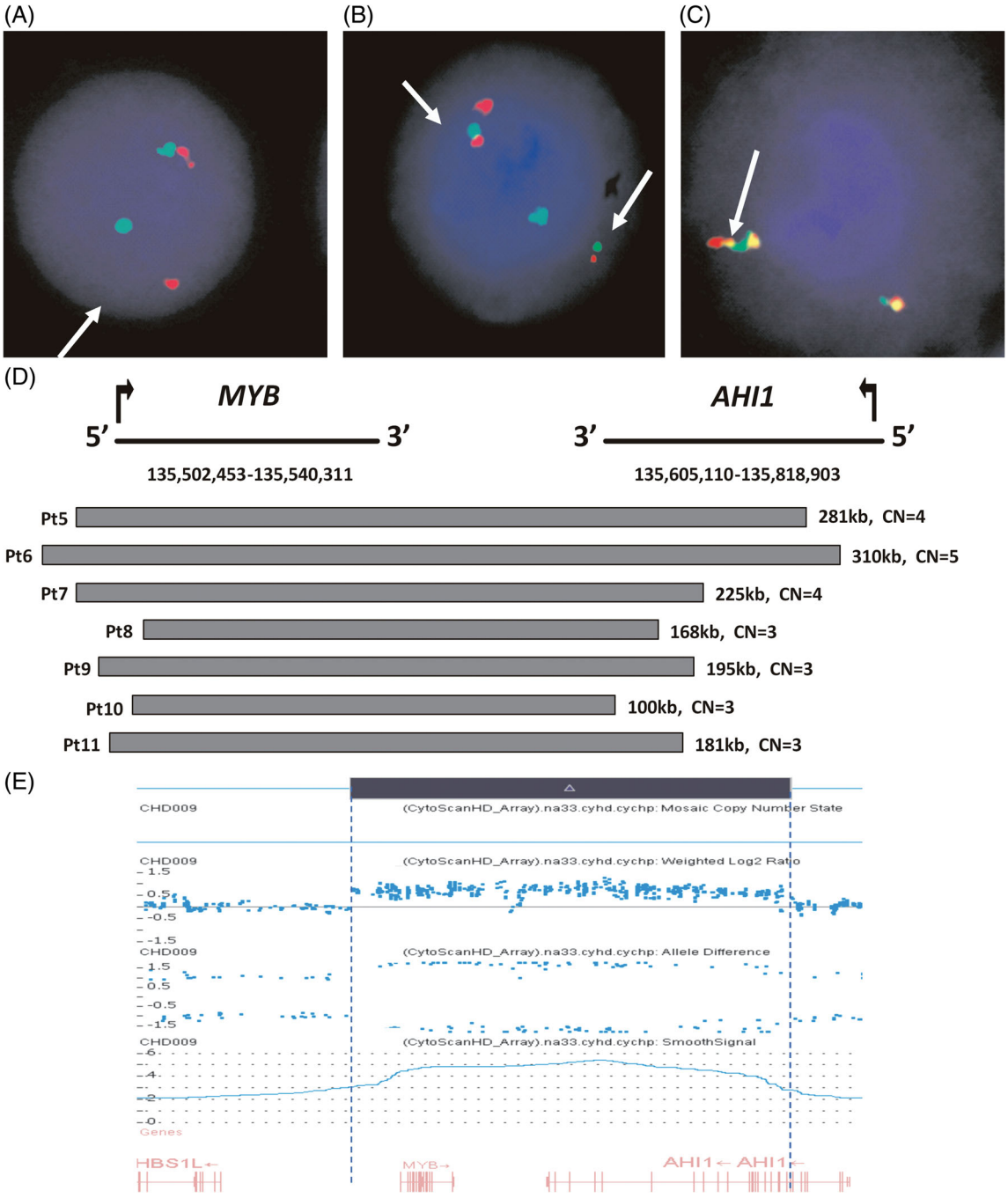

FIGURE 2 Molecular-cytogenetic assessment of *TRB-MYB* and *MYB* tdup. A, Fluorescence in situ hybridization (FISH) with the ZytoVision *MYB* break apart probe detects a *MYB* translocation (split red/green signal) (arrow). B, Double color double fusion FISH experiment with genomic clones for *TRB* (RP11-1220K2/ RP11-556I13) in green, and for *MYB* (RP1-32B1/RP11-1141K5) in orange, shows one orange, one green, and two fusion signals (arrows) confirming the *TRB-MYB* rearrangement (case no.2, Table 3). C, FISH with RP1-32B1/RP11-141K5 shows multiple copies of *MYB* in a local tandem gain (arrow). D, Regions of 6q23 tandem duplication in seven patients who were studied by SNPa (patients' numbers refer to Table 1). E, SNPa profile shows three extra-copies of the 6q23/*MYB* region in case no. 6

MYB-wt cases, 21 patients had *MYB* relative expression values similar to those observed in *MYB*-R cases (≥5.506). *MYB-wt* high expressing cases were unequally distributed into the main genetic subgroups, as they represented the 64% of *TAL/LMO*, 37% of *HOXA*, and 20% of *TLX1/TLX3 cases* (Figure 3C). Accordingly, *TAL/LMO* positive T-ALL showed a significantly higher expression of *MYB* (Mann-Whitney *U* test; $P = .017$) (Figure 3D). Longitudinal analysis of paired diagnostic/remission samples showed that *MYB* expression was between 3- and 140-fold higher at diagnosis than at remission (Figure 3E), displaying a statistically significant difference (Mann-Whitney *U* test; $P = .0006$) (Figure 3F).

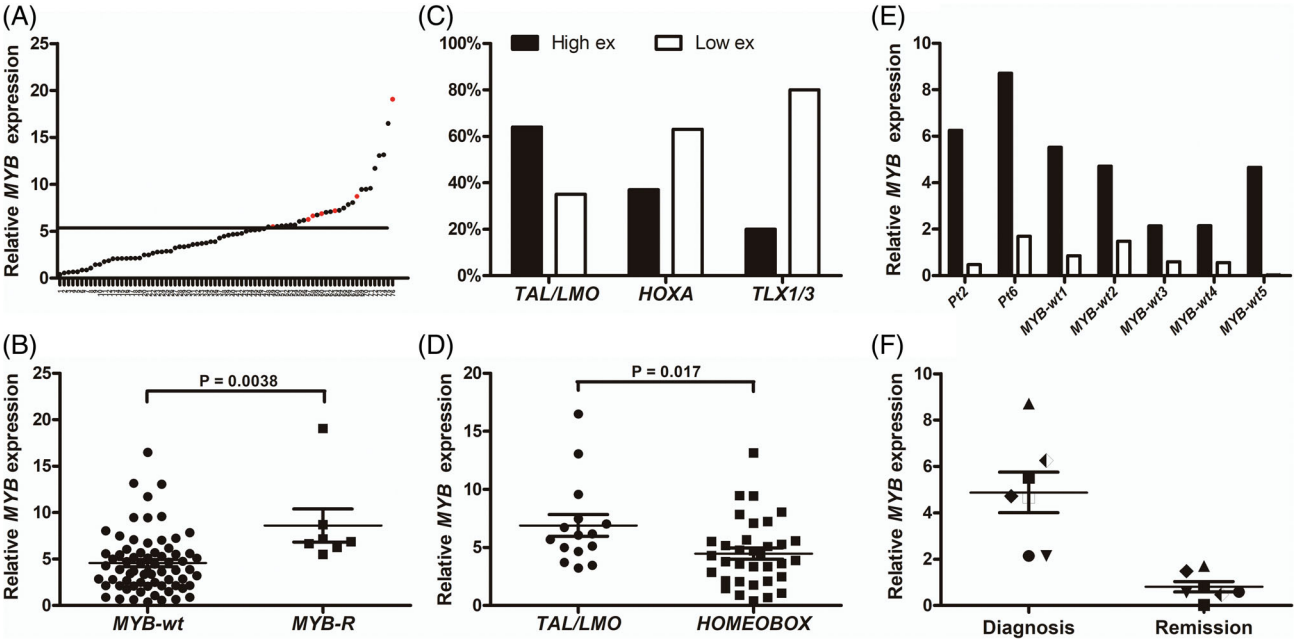

FIGURE 3 *MYB* relative expression analysis in 76 patients with T-cell acute lymphoblastic leukemia (T-ALL). A, A wide range of *MYB* expression has been detected in the 76 patients investigated by quantitative reverse transcription polymerase chain reaction (qRT-PCR); Pt, patient; red dots indicate the 7 cases with rearrangements of *MYB* (*MYB*-R). B, *MYB* expression was significantly higher in *MYB*-R than *MYB*-wt cases (Mann-Whitney *U* test; *P* = .009). C, Distribution of *MYB*-wt T-ALL cases with high (= 21) and low (= 48) *MYB* expression within the main genetic subgroups. D, A significantly higher expression of *MYB* was detected in *TAL/LMO* cases with respect to homeobox-related T-ALL (Mann-Whitney U test, *P* = .017). E, Longitudinal studies on 7 cases with paired diagnostic/remission samples detected a 3-140 higher levels of *MYB* at diagnosis than at remission. F, *MYB* expression was significantly higher at diagnosis than at remission (Mann-Whitney U test; *P* = .0006)

4 | DISCUSSION

First reported in 2007, genomic involvement of *MYB* in T-ALL, was mostly due to *TRB-MYB* and *MYB* tdup, although rare translocations with non-TRB partners, and a recurrent hot-spot mutation, previously reported to perturb the cellular localization and activity of MYB, have been recently detected in pediatric T-ALL.[7,8,10]

We carried out extensive molecular-cytogenetic screening of 331 previously published T-ALL[13] to assess incidence, types, and distribution, of *MYB* abnormalities in pediatric and adult cases. Confirming the low incidence reported in pediatric T-ALL,[10] *MYB* E14 hot-spot mutations were not found in our cases. In line with the first report by Clappier E et al,[8] who reported that *TRB-MYB* specifically occurred in young children, we detected the *TRB-MYB* rearrangement in 1.6% of pediatric T-ALL patients, whose age ranged from 2 to 10 years (Table 3). Conversely, *MYB* tdup occurred in both age groups although it appeared to be slightly more frequent in children than in adults (5.2% vs 2.8%) (Table 3).

This comprehensive cytogenetic study has provided the most reliable approach to investigate *MYB* rearrangements in T-ALL. Although, SNPa cannot identify balanced *TRB-MYB* translocations, it was more sensitive than FISH in detection of *MYB* tdup. As the sensitivity of the two approaches hinged not only on the size of the involved region and clonality, but also on the mechanism of duplication, FISH was unable to detect small *MYB* tdup as contiguous signals cannot not be discriminated, resulting in a lower frequency than previously reported. Furthermore, SNPa also informed on the CNV of *MYB*, revealing that two to four copies of the gene can be involved in the tdup (Figure 2E).

No *MYB*-R were detected among non-ETP cases, in agreement with previous reports. In fact, a former study showed that *TRB-MYB* is one of the genomic rearrangements typically associated with cortical thymocyte arrest.[16] In agreement with these findings, *MYB*-R were frequently associated with *NOTCH1/FBXW7* mutations and *CDKN2AB* deletions, which have a low prevalence in immature T-ALL (Table 3).[17]

Except for three cases that remained undetermined, *MYB*-R were detected within the *HOXA* (47%), *TLX1/NKX2-1* (23%), or *TLX3* (17%) T-ALL subgroups. No *MYB*-R were evident within *TAL/LMO* positive T-ALL (Table 3). Concurrent *MYB*-R alongside established primary abnormalities, suggested that both *TRB-MYB* and *MYB* tdup occurred as secondary rather than primary oncogenic events. Moreover, we also found a biased association with homeobox-related genetic subgroups. These findings are in line with a recent genome-wide sequencing study in pediatric T-ALL, describing a *TRB-MYB* rearrangement in the *TLX3* subgroup, and enrichment of *MYB* tdup in the homeobox-related subgroups.[10] Although, they contrast with the study by Clappier et al, who first described the *TRB-MYB* rearrangement as the biomarker of a specific genetic cluster, characterized by a unique transcriptome profile, distinct from all other T-ALL subtypes.[8]

As previously reported, the relative expression of *MYB* was significantly higher in *MYB*-R than *MYB*-wt cases (Figure 3B). However, high *MYB* expression was also detected in 30% of *MYB*-wt cases, suggesting *MYB* up-regulation is a more frequent event in T-ALL, alternatively related to genomic rearrangements or lesions affecting *cis* and/or *trans* regulatory factors. In keeping with its transcriptional

function, *MYB* high expressing cases were significantly enriched within the *TAL/LMO* subgroup (Mann-Whitney *U* test, *P* = .017) (Figure 3C,D). Remarkably, high levels of *MYB* were strongly associated with the leukemic clone as demonstrated by longitudinal analysis that revealed a significant reduction of *MYB* expression, after treatment, in all cases (Figure 3E,F).

Our study points out the oncogenic role of *MYB* in 30% to 40% of T-ALL.[18] Furthermore, *MYB* scores as an essential dependency in both genome-wide loss-of function RNAi and CRISPR screens in T-ALL and in hematopoietic malignancies more broadly (https://depmap.org/portal), thus emerging a strategic therapeutic target in this subgroup of leukemia.

In conclusion, different mechanisms underlay *MYB* deregulation within distinct settings, indicating gene expression as a unifying diagnostic assay. However, as already used in solid tumors, immunohistochemistry and/or flow cytometry may provide alternative valuable diagnostic tools. The assessment of *MYB* as a prognostic marker, for refining risk stratification of patients, and/or as a target for tailored treatment, requires prospective clinical studies.

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DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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REFERENCES

1. Liu X, Xu Y, Han L, Yi Y. Reassessing the potential of Myb-targeted anti-cancer therapy. *J Cancer*. 2018;9(7):1259-1266. https://doi.org/10.7150/jca.23992.
2. Pattabiraman DR, Gonda TJ. Role and potential for therapeutic targeting of MYB in leukemia. *Leukemia*. 2013;27(2):269-277. https://doi.org/10.1038/leu.2012.225.
3. Ramsay RG, Gonda TJ. MYB function in normal and cancer cells. *Nat Rev Cancer*. 2008;8(7):523-534. https://doi.org/10.1038/nrc2439.
4. Thomas MD, Kremer CS, Ravichandran KS, Rajewsky K, Bender TP. C-Myb is critical for B cell development and maintenance of follicular B cells. *Immunity*. 2005;23(3):275-286. https://doi.org/10.1016/j.immuni.2005.08.005.
5. Nakano K, Uchimaru K, Utsunomiya A, Yamaguchi K, Watanabe T. Dysregulation of c-Myb pathway by aberrant expression of proto-oncogene MYB provides the basis for malignancy in adult T-cell leukemia/lymphoma cells. *Clin Cancer Res*. 2016;22(23):5915-5928. https://doi.org/10.1158/1078-0432.CCR-15-1739.
6. Vicente C, Conchillo A, Pauwels D, et al. MYB overexpression is directly involved in acute myeloid leukemia pathogenesis and could constitute a new therapeutic target for patients with aberrant expression of this gene. *Blood*. 2009;114(22):2402-2402. https://doi.org/10.1182/blood.V114.22.2402.2402.
7. Lahortiga I, De Keersmaecker K, Van Vlierberghe P, et al. Duplication of the MYB oncogene in T cell acute lymphoblastic leukemia. *Nat Genet*. 2007;39(5):593-595. https://doi.org/10.1038/ng2025.
8. Clappier E, Cuccuini W, Kalota A, et al. The C-MYB locus is involved in chromosomal translocation and genomic duplications in human T-cell acute leukemia (T-ALL), the translocation defining a new T-ALL subtype in very young children. *Blood*. 2007;110(4):1251-1261. https://doi.org/10.1182/blood-2006-12-064683.
9. Kawamata N, Zhang L, Ogawa S, et al. Double minute chromosomes containing MYB gene and NUP214-ABL1 fusion gene in T-cell leukemia detected by single nucleotide polymorphism DNA microarray and fluorescence in situ hybridization. *Leuk Res*. 2009;33(4):569-571. https://doi.org/10.1016/j.leukres.2008.07.030.
10. Liu Y, Easton J, Shao Y, et al. The genomic landscape of pediatric and young adult T-lineage acute lymphoblastic leukemia. *Nat Genet*. 2017;49(8):1211-1218. https://doi.org/10.1038/ng.3909.
11. Quelen C, Lippert E, Struski S, et al. Identification of a transforming MYB-GATA1 fusion gene in acute basophilic leukemia: a new entity in male infants. *Blood*. 2011;117(21):5719-5722. https://doi.org/10.1182/blood-2011-01-333013.
12. Suzuki K, Suzuki Y, Hama A, et al. Recurrent MYB rearrangement in blastic plasmacytoid dendritic cell neoplasm. *Leukemia*. 2017;31(7):1629-1633. https://doi.org/10.1038/leu.2017.101.
13. La Starza R, Pierini V, Pierini T, et al. Design of a Comprehensive Fluorescence in situ hybridization assay for genetic Classification of T-cell acute lymphoblastic leukemia. *J Mol Diagn*. 2020;22:629-639. https://doi.org/10.1016/j.jmoldx.2020.02.004.
14. Van Vlierberghe P, Pieters R, Beverloo HB, Meijerink JPP. Molecular-genetic insights in paediatric T-cell acute lymphoblastic leukaemia. *Br J Haematol*. 2008;143(2):153-168. https://doi.org/10.1111/j.1365-2141.2008.07314.x.
15. La Starza R, Barba G, Demeyer S, et al. Deletions of the long arm of chromosome 5 define subgroups of T-cell acute lymphoblastic leukemia. *Haematologica*. 2016;101(8):951-958. https://doi.org/10.3324/haematol.2016.143875.
16. Homminga I, Pieters R, Langerak AW, et al. Integrated transcript and genome analyses reveal NKX2-1 and MEF2C as potential oncogenes in T cell acute lymphoblastic leukemia. *Cancer Cell*. 2011;19(4):484-497. https://doi.org/10.1016/j.ccr.2011.02.008.
17. Belver L, Ferrando A. The genetics and mechanisms of T cell acute lymphoblastic leukaemia. *Nat Rev Cancer*. 2016;16:494-507. https://doi.org/10.1038/nrc.2016.63.
18. Xia M, Zhang Q, Luo M, et al. Regulatory network analysis reveals the oncogenesis roles of feed-forward loops and therapeutic target in T-cell acute lymphoblastic leukemia. *BMC Medical Genomics*. 2019;12(1):8. http://dx.doi.org/10.1186/s12920-018-0469-0.

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