Received: 30 December 2022 | Revised: 9 May 2023 | Accepted: 9 May 2023

DOI: 10.1002/gcc.23151

RESEARCH ARTICLE

WILEY

Increased *MYB* alternative promoter usage is associated with relapse in acute lymphoblastic leukemia

André Fehr[1] | Gustav Arvidsson[2] | Jessica Nordlund[2] | Gudmar Lönnerholm[3] | Göran Stenman[1] | Mattias K. Andersson[1]

[1]Sahlgrenska Center for Cancer Research, Department of Pathology, University of Gothenburg, Gothenburg, Sweden

[2]Department of Medical Sciences and Science for Life Laboratory, Uppsala University, Uppsala, Sweden

[3]Department of Women's and Children's Health, Uppsala University, Uppsala, Sweden

Correspondence
Mattias K. Andersson, Sahlgrenska Center for Cancer Research, Department of Pathology, University of Gothenburg, Box 425, SE-405 30 Gothenburg, Sweden.
Email: mattias.andersson@llcr.med.gu.se

Funding information
Barncancerfonden; Cancerfonden; Sjöbergstiftelsen; Vetenskapsrådet

Abstract

Therapy-resistant disease is a major cause of death in patients with acute lymphoblastic leukemia (ALL). Activation of the *MYB* oncogene is associated with ALL and leads to uncontrolled neoplastic cell proliferation and blocked differentiation. Here, we used RNA-seq to study the clinical significance of *MYB* expression and *MYB* alternative promoter (TSS2) usage in 133 pediatric ALLs. RNA-seq revealed that all cases analyzed overexpressed *MYB* and demonstrated *MYB* TSS2 activity. qPCR analyses confirmed the expression of the alternative *MYB* promoter also in seven ALL cell lines. Notably, high *MYB* TSS2 activity was significantly associated with relapse ($p = 0.007$). Moreover, cases with high *MYB* TSS2 usage showed evidence of therapy-resistant disease with increased expression of ABC multidrug resistance transporter genes (e.g., *ABCA2*, *ABCB5*, and *ABCC10*) and enzymes catalyzing drug degradation (e.g., *CYP1A2*, *CYP2C9*, and *CYP3A5*). Elevated *MYB* TSS2 activity was further associated with augmented KRAS signaling ($p < 0.05$) and decreased methylation of the conventional *MYB* promoter ($p < 0.01$). Taken together, our results suggest that *MYB* alternative promoter usage is a novel potential prognostic biomarker for relapse and therapy resistance in pediatric ALL.

KEYWORDS
acute lymphoblastic leukemia, alternative promoter, MYB, MYB TSS2, relapse, therapy resistance

1 | INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common childhood cancer and a leading cause of cancer-related death in children.[1] Although dramatic improvements in survival have been made through multimodal risk-based therapy, current risk allocation schemes are inadequate. For example, children that are classified as low-risk do relapse.[2] Relapse of drug-resistant disease occurs in up to 20% of cases and most of these patients succumb to their disease.[1,3] Hence, new prognostic biomarkers that may improve patient stratification, as well as novel treatments for relapsed/refractory ALL, are needed.

MYB encodes a master transcriptional regulator involved in cell proliferation and stem cell renewal.[4–7] MYB is an oncogenic driver in the majority of leukemia and is overexpressed by various molecular mechanisms.[8] In ALL, the *MYB* gene is activated by gene duplication, gene fusion, or point mutation.[9–12] In addition, *MYB* is a critical downstream target of KMT2A-fusions and is required for BCR::ABL1-driven

leukemogenesis.[13–15] The *MYB* gene and its downstream effectors are thus potential therapeutic targets in pediatric ALL.[8,16]

The transcriptional regulation of *MYB* is complex with an alternative *MYB* promoter located in intron 1 that is differentially activated between normal and leukemic cells.[17,18] Expression of the alternative *MYB* promoter was recently demonstrated also in primary adenoid cystic carcinomas.[19] Notably, ectopic expression of N-terminally truncated forms of MYB, corresponding to alternative promoter usage, resulted in an altered MYB downstream target gene repertoire. In this study, we have investigated the clinical significance of *MYB* alternative promoter activity in a large, well-characterized cohort of 133 pediatric ALLs with clinical follow-up data. To our knowledge, this is the first study evaluating *MYB* alternative promoter activity in leukemia patient samples.

2 | MATERIALS AND METHODS

2.1 | Patient material

The ALL patient cohort has previously been described in detail.[20] A summary of clinical, cytogenetic, and molecular characteristics is shown in Table S1. The cohort includes 115 cases of pediatric B-ALL, 18 cases of pediatric T-ALL, and normal control lymphocytes isolated from peripheral blood from five healthy Swedish donors. Clinical follow-up data were available for all cases. The study was approved by the regional ethics board in Uppsala, Sweden (D-No: 2013–237). The study was done in accordance with the Helsinki Declaration.

2.2 | Cell culture

The B-ALL cell lines[21–25] REH (DSMZ no. ACC-22), 697 (DSMZ no. ACC-42), SUP-B15 (DSMZ no. ACC-389), SEM (DSMZ no. ACC-546), NALM16 (DSMZ no. ACC-680), and T-ALL cell lines[26–28] MOLT-4 (ATCC no. CRL-1582), CCRF-CEM (ATCC no. CCL-119), P12-ICHIKAWA (DSMZ no. ACC-34), RPMI-8402 (DSMZ no. ACC-290) were obtained from DSMZ or ATCC. Cells were maintained in RPMI-1640 medium with GlutaMAX, 10% or 20% FBS, and 1% penicillin–streptomycin (Thermo Fisher Scientific, Waltham, MA) according to the instructions of the suppliers. Cells were kept at 37°C and 5% CO_2 in a humidified incubator.

2.3 | RNA-seq, targeted DNA sequencing, and DNA methylation analysis

Total RNA was previously extracted from B- and T-ALLs and control lymphocytes, and strand-specific RNA libraries were constructed, sequenced, and aligned to the human reference genome GRCh37 as described.[20] Read counts for genes and individual *MYB* exons were summarized with featureCounts.[29] Counts of reads aligning to *MYB* exon 1 (ENSE00002174682) and exon 2 (ENSE00002169478) were normalized for exon length and the exon 2/exon 1 expression ratio was used to estimate *MYB* alternative promoter activity. Full-length *MYB* expression was determined by the number of reads assigned to *MYB* exons and normalized for total reads per sample and *MYB* exon lengths. Unsupervised hierarchical clustering, heatmap visualization, and differential gene expression analysis were done with *pheatmap* of R (v. 3.5.3) and DESeq2.[30] Genes included in the analysis had a minimum of 10 read counts in at least six samples. Genes with a log fold change of at least 2 and an adjusted *p* value (Benjamini–Hochberg) of <0.01 were considered differentially expressed. Gene set enrichment analysis (GSEA) was done with the GSEA software (v.4.2.2)[31] and gene ontologies were analyzed with ToppGene.[32] Venn diagrams were created using BioVenn.[33] Methylation data for probes located within and proximal to the 5′ part of *MYB* were available in a previously published data set[34] for the 18/18 T-ALLs and 18/20 B-ALLs analyzed with differential gene expression. *KRAS* mutation status was available for 12/18 T-ALLs and 12/20 B-ALLs from previously reported targeted sequencing data.[35]

2.4 | Quantitative real-time PCR (qPCR)

Total RNA was isolated from ALL cell lines with the RNeasy Micro Kit (Qiagen, Hilden, Germany) and reverse transcribed with the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA). The *MYB* alternative promoter activity in ALL cell lines was estimated by Taqman gene expression assays (Thermo Fisher Scientific) specific for *MYB* exons 1–2 (Hs00920554_m1) and 2–3 (Hs00920555_m1). The qPCR efficiencies[36] of *MYB* exons 1–2 and *MYB* exons 2–3 probes were determined by serial dilution. The ratios of *MYB* exons 2–3 to *MYB* exons 1–2 expression were estimated for each sample by incorporating qPCR efficiencies as a measure of *MYB* TSS2 activity. Taqman probes for *MYB* (Hs00920555_m1) and *UBC* (Hs01871556_s1), as a reference gene, were used to estimate the total *MYB* expression.

2.5 | Statistics

Mann–Whitney test, analysis of variance (ANOVA), independent samples t-test, Pearson correlation, Fisher's exact test, logrank test, and Kaplan–Meier survival analysis were done with Prism v.9 (GraphPad Software, San Diego, CA). Multivariate analysis using Cox regression was performed with SPSS Statistics v.28 (IBM, Armonk, NY). Confirmation of the proportional hazards assumption and linearity of Martingale and deviance residuals was done with the *survival* package of R.

3 | RESULTS

3.1 | Increased *MYB* alternative promoter usage is associated with relapse in pediatric ALL

Using RNA-seq, we studied *MYB* alternative promoter (TSS2) activity in primary samples from 133 pediatric ALL patients by calculating the

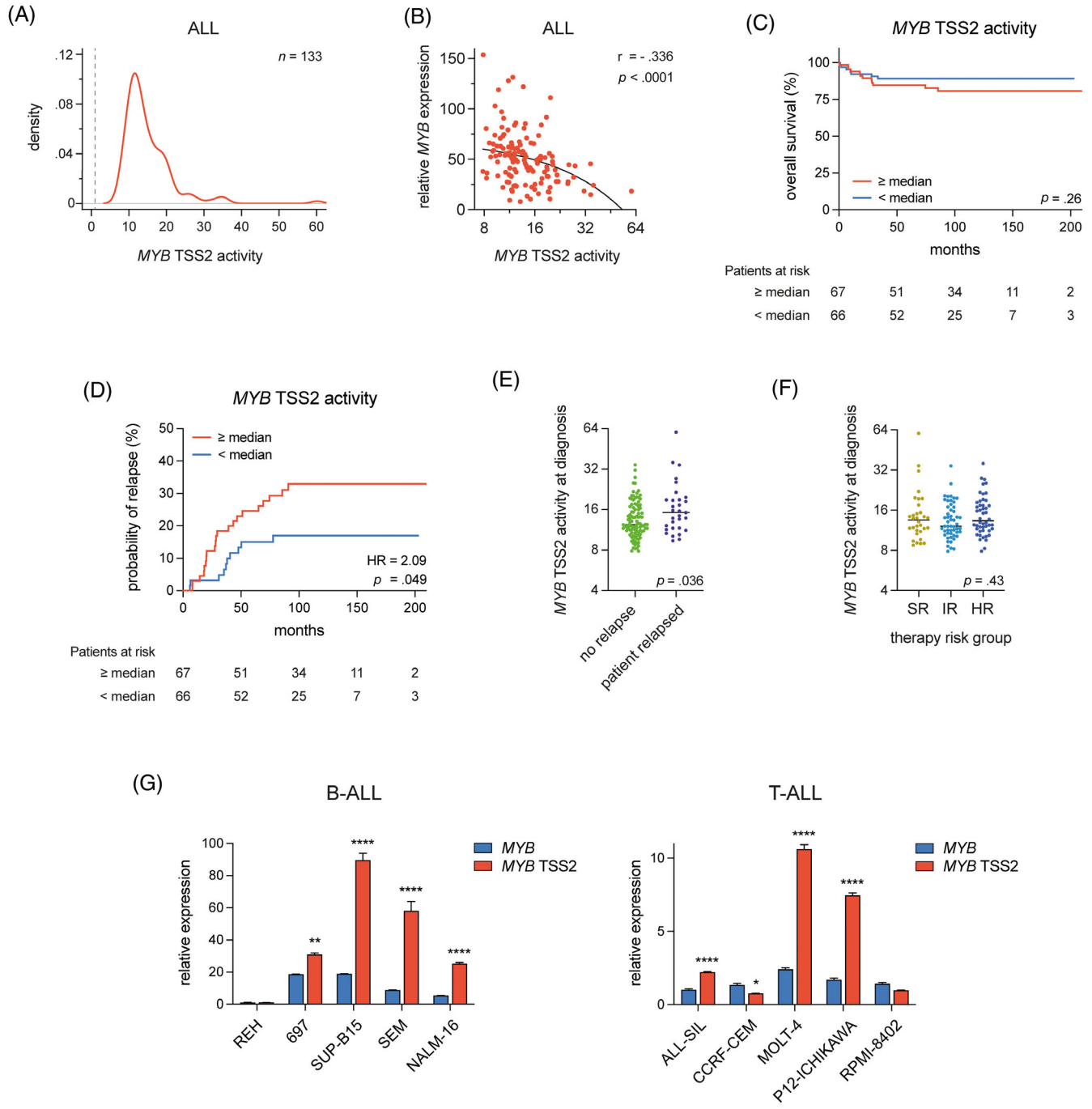

FIGURE 1 Clinical significance of *MYB* TSS2 usage in pediatric acute lymphoblastic leukemia (ALL). (A) Kernel density estimation of *MYB* TSS2 activity in 133 pediatric ALLs. (B) Pearson correlation of relative *MYB* expression and *MYB* TSS2 activity in 133 ALLs. (C) Kaplan–Meier analysis of overall survival in cases grouped by median *MYB* TSS2 activity. (D) Estimation of the risk of relapse in ALL cases grouped by median *MYB* TSS2 activity using Kaplan–Meier analysis. The hazard ratio (HR) was estimated by the logrank test. (E) Comparison of *MYB* TSS2 activity between ALL patients that relapsed and did not relapse during follow-up using the Mann–Whitney test. (F) Comparison of *MYB* TSS2 activity between therapy risk groups of the ALL patients using the one-way analysis of variance (ANOVA) test. SR, standard risk; IR, intermediate risk; HR, high risk. (G) *MYB* expression and *MYB* TSS2 activity in ALL cell lines. Two-way ANOVA test was used to evaluate differences between *MYB* TSS2 activity and total *MYB* expression (*$p < 0.05$; **$p < 0.01$; ****$p < 0.0001$).

ratio of *MYB* exon 2 to exon 1 expression for each sample. The *MYB* TSS2 activity showed a right-skewed distribution using kernel density estimation (Figure 1A) with all patient samples having an exon2/exon1 ratio higher than 7 and a median TSS2 activity of 12.96. This implies a prominent alternative promoter usage in all cases. Comparison of *MYB* TSS2 activity against full-length *MYB* expression revealed a slight but significant inverse correlation (Figure 1B), indicating differential regulation of the two *MYB* promoters. Next, we asked if *MYB*

Variable	HR	95% CI for HR	*p* value
MYB TSS2 activity	1.049	1.013–1.086	0.007
Age	0.845	0.740–0.965	0.013
White blood cell count	1.002	0.998–1.005	0.289
Cytogenetic/immunophenotypic subtype			
11q23/KMT2A	1.068	0.233–4.890	0.933
t(12;21)	0.578	0.155–2.158	0.415
t(9;22)	9.368	1.957–44.851	0.005
HeH	0.554	0.205–1.493	0.243
T-ALL	1.358	0.272–6.783	0.709
B-ALL other	reference	reference	reference

TABLE 1 Multivariate analysis of the risk of relapse in pediatric acute lymphoblastic leukemia (ALL) ($n = 133$) estimated by Cox regression.

Abbreviations: CI, confidence interval; HeH, high hyperdiploidy; HR, hazard ratio.

TSS2 usage was associated with overall survival and/or the probability of relapse in our patient cohort. Kaplan–Meier analyses demonstrated that cases with equal or above median *MYB* TSS2 activity showed a trend toward shorter overall survival, but the difference compared to cases with TSS2 activity below the median was not statistically significant (Figure 1C). In contrast, high *MYB* TSS2 activity was significantly associated with relapse ($p = 0.049$) and cases with high TSS2 activity showed a two-fold increased risk of recurrence (HR = 2.09, 95% CI: 1.04–4.23) (Figure 1D). *MYB* TSS2 activity was significantly higher in samples from patients that later relapsed (Figure 1E) and was independent of the therapy risk group (Figure 1F). Moreover, the magnitude of *MYB* TSS2 activity was a significant factor for relapse in a multivariate analysis ($p = 0.007$) that also included age, cytogenetic/immunophenotypic subtype, and white blood cell count (Table 1). The risk of relapse increased by approximately 5% per additional unit of *MYB* TSS2 activity. Taken together, our results indicate that *MYB* TSS2 activity may be of prognostic value in pediatric ALL. Notably, total *MYB* gene expression was upregulated in all cases compared to control lymphocytes but was neither associated with overall survival nor relapse in our cohort (Figure S1). Using qPCR, we also assayed *MYB* expression and *MYB* TSS2 activity in cultured ALL cells derived from the B- and T-cell lineages (Figure 1G). Increased *MYB* TSS2 activity was found in 7/10 ALL cell lines, indicating that *MYB* alternative promoter activity occurs in a significant number of cases also in vitro.

3.2 | *MYB* TSS2 usage is independent of cytogenetic and immunophenotypic subtype in ALL

Since cytogenetic abnormalities and immunophenotype are important for prognostication and treatment stratification of ALL,[1] we investigated *MYB* TSS2 activity in the previously defined subtypes[20] of our cohort (Figure 2A). There were no significant differences between ALL subtypes in terms of *MYB* TSS2 usage, indicating that *MYB* TSS2 activity is not related to the diagnostic subtype and that it is an independent biomarker of relapse (Table 1). Interestingly, the T-ALL cases seemingly separated into two subgroups in terms of TSS2 activity (Figure 2A). These subgroups became clear when assessing *MYB* TSS2 activity in the T-ALL cases specifically (Figure 2B). All T-ALL cases with relapse belonged to the *MYB* TSS2 high group (Figure 2C), suggesting an association between *MYB* TSS2 usage and relapse in this ALL subtype. To investigate potential differences in clinical and genetic parameters between T-ALL cases with high or low *MYB* TSS2 activity, we analyzed sex, age, white blood cell count, karyotype, the presence of gene fusions, and mutations in the NOTCH pathway (Figure 2D, E). These analyses revealed that abnormal karyotypes and fusion oncogenes were less common in the *MYB* TSS2 high group.

3.3 | Different levels of *MYB* TSS2 activity are associated with distinct gene expression profiles in ALL

To investigate if global gene expression was associated with the level of *MYB* TSS2 activity in T-ALL, we analyzed the transcriptomic profiles of the 18 T-ALL cases. Unsupervised hierarchical clustering revealed that the T-ALL cases formed two distinct clusters and that one of these contained only cases with high TSS2 usage (Figure 3A, left panel). The other cluster included all cases with low TSS2 usage as well as two cases belonging to the TSS2 high group. Notably, these two cases had the lowest TSS2 activity of all cases in the high group, indicating that *MYB* TSS2 activity above a certain threshold may be associated with specific gene expression patterns. To study if similar patterns were present also in B-ALL, we selected the 10 B-ALL cases with the highest *MYB* TSS2 activity and the 10 cases with the lowest activity and analyzed their global gene expression profiles. Analogous to T-ALL, the B-ALL cases formed two clusters; one with only TSS2 high cases and one including all TSS2 low cases (Figure 3A, right panel). As for T-ALL, two TSS2 high cases were found in the TSS2 low cluster and these cases showed the lowest TSS2 activation in the TSS2 high group. Taken together, our data indicate a strong association between gene expression profiles and *MYB* TSS2 activity in ALL, irrespective of immunophenotype.

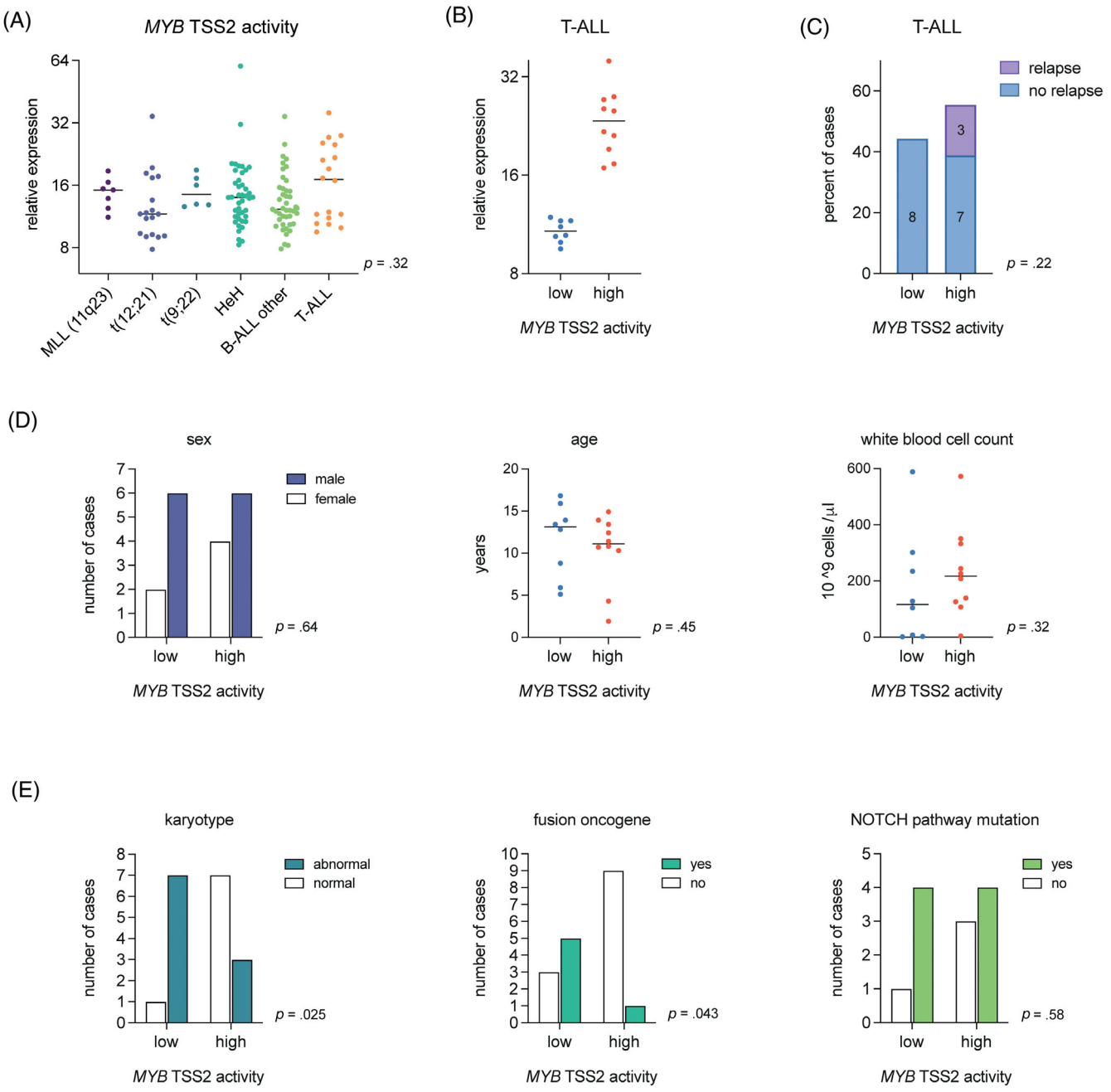

FIGURE 2 *MYB* TSS2 activity in acute lymphoblastic leukemia (ALL) subtypes. (A) Comparison of *MYB* TSS2 activity in cytogenetic and immunophenotypic subtypes of ALL using the analysis of variance test. (B) *MYB* TSS2 activity in 18 T-ALL cases. (C) Comparison of the relapse frequency for *MYB* TSS2 high and low groups of T-ALL. (D) Comparison of clinical parameters between *MYB* TSS2 high and low T-ALLs. (E) Differences in genetic characteristics of *MYB* TSS2 high and low T-ALL cases. NOTCH pathway mutations included mutations in *NOTCH1-4* and *FBXW7*. The Fisher's exact test or the Mann–Whitney test were used to estimate statistical differences in C–E. HeH, high hyperdiploidy.

3.4 | ALLs with high *MYB* TSS2 usage show decreased expression of conventional MYB target genes and increased expression of genes involved in KRAS signaling and transmembrane transport

Next, we investigated differences in MYB target gene activation between ALLs with high and low TSS2 activity. We used GSEA to compare the RNA-seq data with a previously published dataset of direct MYB target genes in hematopoietic progenitor cells.[37] Conventional MYB target gene expression was specifically enriched in TSS2 low cases (Figure 3B), suggesting a different downstream target gene repertoire for *MYB* TSS2-encoded proteins in ALL.

We then studied which other genes and pathways that were upregulated in ALL cases with high *MYB* TSS2 usage. GSEA demonstrated that both T-ALLs and B-ALLs with high *MYB* TSS2 activity showed upregulation of genes involved in KRAS signaling (Figure 3C). To investigate if the

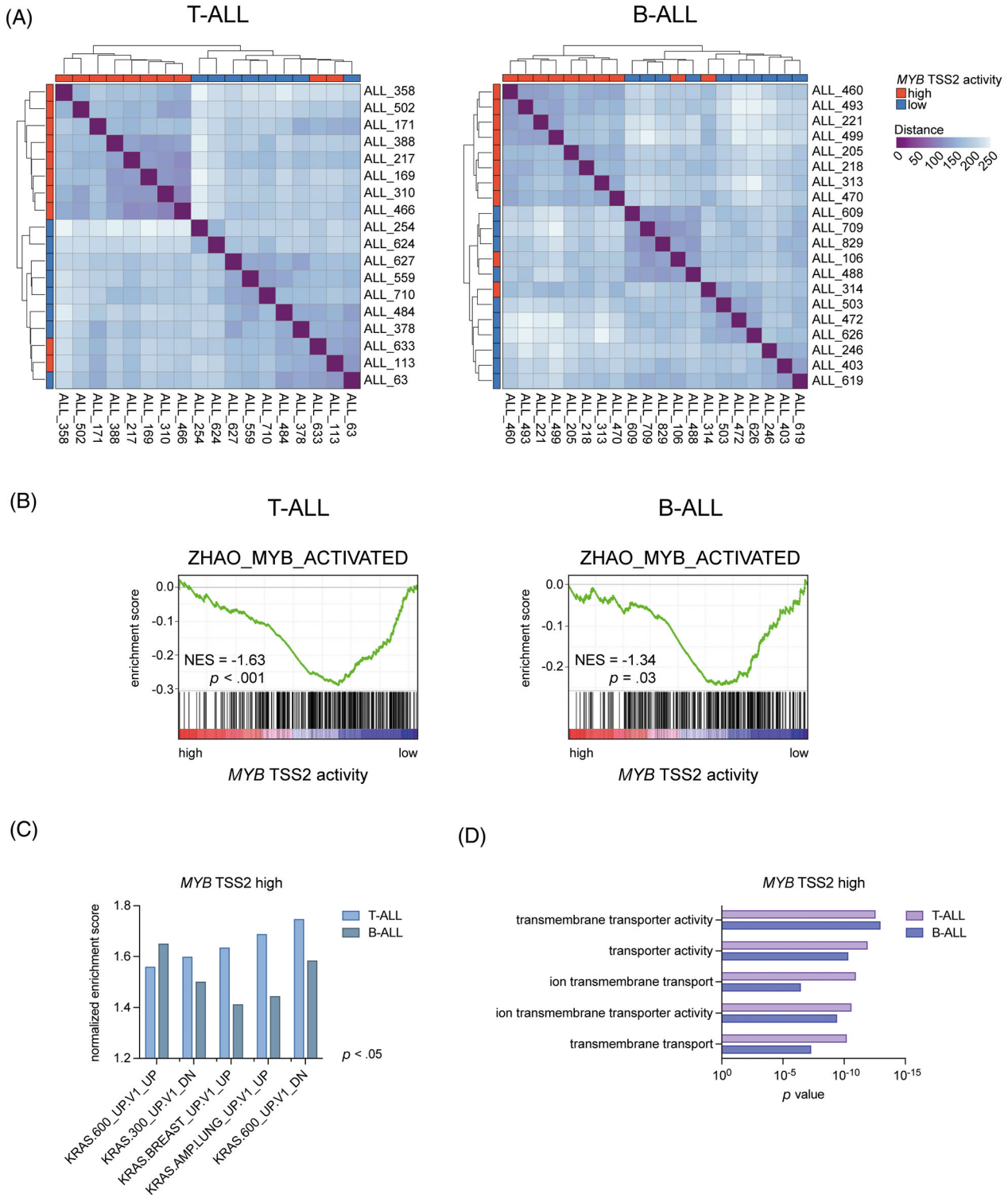

FIGURE 3 RNA-seq analysis of global gene expression in pediatric acute lymphoblastic leukemia (ALL). (A) Hierarchical clustering and distance matrices of RNA-seq global gene expression in *MYB* TSS2 high and low cases of T- and B-ALL. (B) GSEA of direct MYB target genes in *MYB* TSS2 high and low cases. (C) Significantly enriched gene sets in T- and B-ALL cases with high *MYB* TSS2 activity using GSEA. (D) Significant gene ontologies in T- and B-ALL cases with high *MYB* TSS2 activity.

increased KRAS signaling in the *MYB* TSS2 high cases was associated with *KRAS* mutations, we analyzed previously published targeted sequence data for a subset of these cases.[35] No *KRAS* mutations were found in neither *MYB* TSS2 high nor low cases, suggesting that alternative mechanisms for KRAS pathway activation are at play in *MYB* TSS2 high cases. Interestingly, gene ontology analysis revealed that cases with high *MYB* TSS2 usage had an increased expression of genes encoding transmembrane transporters (Figure 3D). In particular, increased expression of

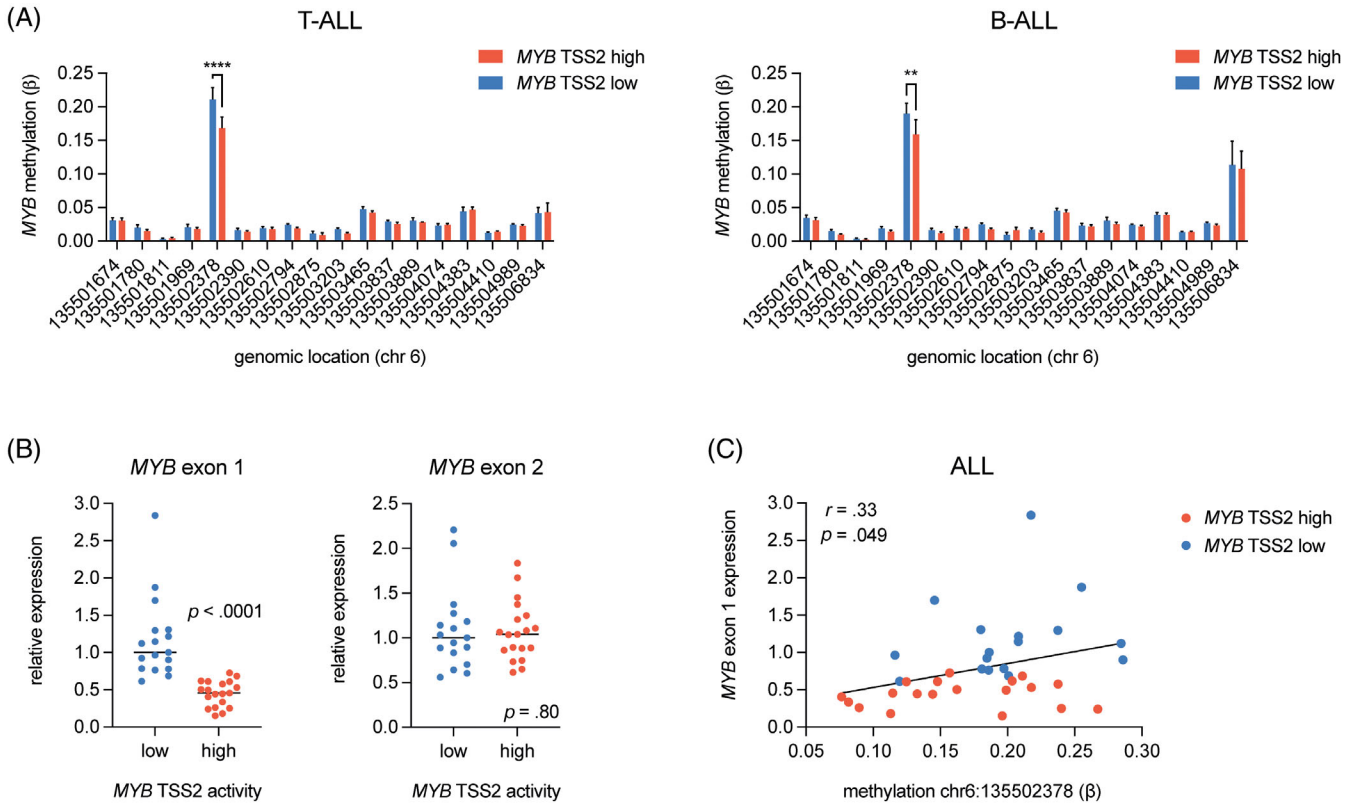

FIGURE 4 DNA methylation analysis of CpG sites proximal to and within the *MYB* gene. (A) Comparison of CpG methylation within and proximal to the *MYB* gene in T- and B-ALL between cases with high and low *MYB* TSS2 activity using the Mann–Whitney test. (B) Comparison of the relative expression of *MYB* exons 1 and 2 between ALL cases with high and low *MYB* TSS2 activity using the Mann–Whitney test. (C) Pearson correlation of *MYB* exon 1 expression and methylation at chr6:135502378 (GRCh37) in ALL.

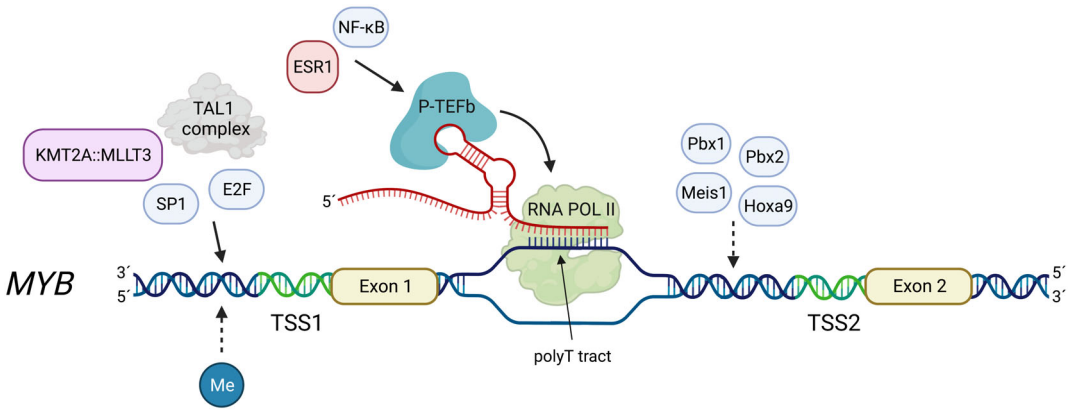

FIGURE 5 A schematic overview of positive regulators of *MYB* transcription. In KMT2A-rearranged leukemia, for example, KMT2A::MLLT3, the KMT2A-fusion protein binds the core *MYB* promoter and activates transcription through the recruitment of several transcriptional regulators.[40] The TAL1 complex, E2F factors, and SP1 activate *MYB* transcription from the conventional *MYB* promoter in T-ALL cells.[41,42] In the current model of *MYB* transcription elongation control, the polyT tract in intron 1 causes RNA polymerase II stalling, RNA (red) stem-loop formation, and attenuation of transcription elongation. *MYB* transcription elongation arrest is relieved by ESR1 in ER + breast cancer cells[43] and by NF-κB factors in colon cancer cells through interaction with P-TEFb.[44] Pbx1, Pbx2, Meis1, and Hoxa9 bind the alternative *MYB* promoter located in intron 1 in mouse hematopoietic cells.[18] In our study, we found evidence of positive regulation of *MYB* exon 1 expression by methylation of the conventional promoter at chr6:135,502,378 (GRCh37). ESR1, estrogen receptor alpha; Me, methylation; P-TEFb, positive transcription elongation factor b; RNA POL II, RNA polymerase II; TSS, transcription start site. Created with BioRender.com.

genes encoding ABC drug transporters (e.g. *ABCA2*, *ABCB5*, and *ABCC10*) and enzymes catalyzing drug degradation (e.g. *CYP1A2*, *CYP2C9*, and *CYP3A5*) were found in *MYB* TSS2 high cases. This may lead to an increased risk of development of drug-resistant disease.

3.5 | Decreased methylation of the conventional *MYB* promoter is seen in ALL cases with high *MYB* TSS2 usage

To gain further insights into the mechanism behind differential *MYB* TSS2 regulation, we analyzed the methylation status of 18 CpG sites located within the conventional *MYB* promoter and within the first exon and intron in 18 B-ALL and 18 T-ALL cases (Figures 3 and 4). The CpG site located on chromosome 6 at 135,502,378 (GRCh37) in the core *MYB* promoter was markedly enriched for methylation compared with other analyzed sites (Figure 4A). Notably, *MYB* TSS2 high cases of both B- and T-ALL had significantly less methylation at this site than *MYB* TSS2 low cases. To investigate a putative association between *MYB* regulation and methylation, we first analyzed the expression of exon 1 and exon 2 in *MYB* TSS2 high and low cases (Figure 4B). *MYB* exon 1 expression was significantly lower in cases with high *MYB* TSS2 activity, whereas exon 2 expression was similar between the groups. Furthermore, exon 1 expression was significantly correlated with methylation at 6:135,502,378 (Figure 4C). These results suggest that the total amount of *MYB* exon 2 transcripts is similar in *MYB* TSS2 high and low cases but that *MYB* TSS2 high cases have significantly more transcripts initiated from TSS2 and less transcripts initiated from transcription start site 1 (TSS1) in the conventional promoter. Taken together, our results suggest that the expression of transcripts containing *MYB* exon 1 may be partly regulated by DNA methylation of a regulatory element located in the core promoter.

4 | DISCUSSION

MYB encodes an oncogenic master transcription factor that is activated and overexpressed in the majority of human leukemias.[8] Here, we investigated the clinical significance of *MYB* overexpression and alternative promoter usage in 133 pediatric ALLs. *MYB* was overexpressed in all cases and *MYB* TSS2 promoter activity was an independent predictor of ALL relapse in a multivariate analysis. Interestingly, *MYB* TSS2 activity was not related to the different therapy risk groups of the pediatric ALL patients. Our findings, thus, indicate that *MYB* TSS2 activity could potentially be used to pinpoint cases with a higher risk of relapse in the standard and intermediate treatment groups. Future prospective studies will determine whether *MYB* TSS2 activity may complement current prognostic factors, such as minimal residual disease,[38] in ALL prognostication. *MYB* TSS2 analysis could potentially be incorporated in clinical routine by using qPCR with *MYB*-specific primers or RNA-seq. In many countries, efforts are now made at the national level to include RNA-seq of acute leukemia in clinical routine.[39]

In this study, *MYB* TSS2 activity was not associated with cytogenetic or immunophenotypic subtypes, which implies subtype-independent mechanisms of *MYB* regulation in ALL. We found an inverse correlation between *MYB* TSS1 and TSS2 activity and an association between DNA methylation of the conventional *MYB* promoter and expression of transcripts containing exon 1. In the ALL cases with lower promoter methylation, we detected an enrichment of transcripts starting with exon 2 and fewer transcripts starting with exon 1. Our results suggest that the CpG site at 6:135,502,378 is, at least partly, involved in the recruitment of factors regulating the expression of the *MYB* gene. Additional studies are needed to clarify the mechanisms regulating the conventional and the alternative *MYB* promoter and potential crosstalk between these two in ALL. A schematic overview of known regulators of *MYB* transcription is shown in Figure 5.

Expression of truncated variants of MYB, corresponding to alternative promoter usage, has previously been shown to result in altered MYB-regulated downstream gene expression.[19] In our study, high *MYB* TSS2 activity was associated with decreased expression of conventional MYB target genes and increased expression of genes involved in KRAS signaling and transmembrane transport. Notably, cases with DNA sequencing data lacked *KRAS* mutations, suggesting that signaling downstream of KRAS, and not the protein itself, is activated in *MYB* TSS2 high cases. Importantly, RAS pathway activation has previously been linked to relapse and therapy resistance in ALL,[45] and our data further support this association. We also detected increased expression of genes encoding ABC drug transporters in *MYB* TSS2 high cases. Since upregulation of these genes correlates with therapy resistance and poor prognosis in ALL,[46] this could be another reason for the frequent relapse of cases with high *MYB* TSS2 activity.

A limitation of our work is the restricted number of cases for certain ALL subtypes (Table S1). Another limitation is the lack of detailed mechanistic studies of *MYB* TSS2 regulation. However, we show that decreased DNA methylation was correlated with the ratio of transcripts originating from *MYB* TSS1 and TSS2. High *MYB* TSS2 activity was more frequently found in T-ALL cases with normal karyotype and without detectable fusion oncogenes, further supporting a role for epigenetic mechanisms in *MYB* TSS2 regulation in ALL. We also show that *MYB* TSS2 activity occurs in cultured ALL cell lines, which opens up for in vitro studies of alternative promoter regulation and drug resistance.

In summary, we present the first study of *MYB* expression and *MYB* alternative promoter usage in primary samples of pediatric ALL. *MYB* was overexpressed in all cases and *MYB* alternative promoter activity was associated with an increased risk of relapse, conceivably mediated by augmented KRAS signaling and transmembrane transporter activity. Our results indicate that *MYB* alternative promoter expression is a potential novel prognostic biomarker for relapse in pediatric ALL.

FUNDING INFORMATION

This work was funded by the Swedish Childhood Cancer Fund, the Sjöberg Foundation, the Swedish Cancer Society, and the Swedish Research Council.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

RNA sequencing data are available for academic purposes upon reasonable request by contacting the corresponding author. The patient/parent consent does not cover the public deposition of data that can be used for the large-scale determination of germline variants.

PATIENT CONSENT

The guardians of the pediatric ALL patients provided written or oral consent to the study.

ORCID

André Fehr https://orcid.org/0000-0002-2657-1392
Göran Stenman https://orcid.org/0000-0003-1017-7363
Mattias K. Andersson https://orcid.org/0000-0001-6391-2048

REFERENCES

1. Hunger SP, Mullighan CG. Acute lymphoblastic leukemia in children. *N Engl J Med*. 2015;373(16):1541-1552.
2. Teachey DT, Hunger SP. Predicting relapse risk in childhood acute lymphoblastic leukaemia. *Br J Haematol*. 2013;162(5):606-620.
3. Roberts KG. Genetics and prognosis of ALL in children vs adults. *Hematology Am Soc Hematol Educ Program*. 2018;2018(1):137-145.
4. Ramsay RG, Gonda TJ. MYB function in normal and cancer cells. *Nat Rev Cancer*. 2008;8(7):523-534.
5. Andersson MK, Afshari MK, Andren Y, Wick MJ, Stenman G. Targeting the oncogenic transcriptional regulator MYB in adenoid cystic carcinoma by inhibition of IGF1R/AKT signaling. *J Natl Cancer Inst*. 2017;109(9):djx017.
6. Fuglerud BM, Lemma RB, Wanichawan P, Sundaram AYM, Eskeland R, Gabrielsen OS. A c-Myb mutant causes deregulated differentiation due to impaired histone binding and abrogated pioneer factor function. *Nucleic Acids Res*. 2017;45(13):7681-7696.
7. Andersson MK, Mangiapane G, Nevado PT, et al. ATR is a MYB regulated gene and potential therapeutic target in adenoid cystic carcinoma. *Oncogenesis*. 2020;9(1):5.
8. Pattabiraman DR, Gonda TJ. Role and potential for therapeutic targeting of MYB in leukemia. *Leukemia*. 2013;27(2):269-277.
9. 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.
10. 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.
11. 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.
12. Bardelli V, Arniani S, Pierini V, et al. MYB rearrangements and overexpression in T-cell acute lymphoblastic leukemia. *Genes Chromosomes Cancer*. 2021;60(7):482-488.
13. Hess JL, Bittner CB, Zeisig DT, et al. c-Myb is an essential downstream target for homeobox-mediated transformation of hematopoietic cells. *Blood*. 2006;108(1):297-304.
14. Zuber J, Rappaport AR, Luo W, et al. An integrated approach to dissecting oncogene addiction implicates a Myb-coordinated self-renewal program as essential for leukemia maintenance. *Genes Dev*. 2011;25(15):1628-1640.
15. Waldron T, De Dominici M, Soliera AR, et al. c-Myb and its target Bmi1 are required for p190BCR/ABL leukemogenesis in mouse and human cells. *Leukemia*. 2012;26(4):644-653.
16. Tejera Nevado P, Tesan Tomic T, Atefyekta A, Fehr A, Stenman G, Andersson MK. Synthetic oleanane triterpenoids suppress MYB oncogene activity and sensitize T-cell acute lymphoblastic leukemia cells to chemotherapy. *Front Oncol*. 2023;13:1126354.
17. Jacobs SM, Gorse KM, Westin EH. Identification of a second promoter in the human c-myb proto-oncogene. *Oncogene*. 1994;9(1):227-235.
18. Dasse E, Volpe G, Walton DS, et al. Distinct regulation of c-myb gene expression by HoxA9, Meis1 and Pbx proteins in normal hematopoietic progenitors and transformed myeloid cells. *Blood Cancer J*. 2012;2(6):e76.
19. Frerich CA, Sedam HN, Kang H, Mitani Y, El-Naggar AK, Ness SA. N-terminal truncated Myb with new transcriptional activity produced through use of an alternative MYB promoter in salivary gland adenoid cystic carcinoma. *Cancers (Basel)*. 2019;12(1):45.
20. Marincevic-Zuniga Y, Dahlberg J, Nilsson S, et al. Transcriptome sequencing in pediatric acute lymphoblastic leukemia identifies fusion genes associated with distinct DNA methylation profiles. *J Hematol Oncol*. 2017;10(1):148.
21. Rosenfeld C, Goutner A, Choquet C, et al. Phenotypic characterisation of a unique non-T, non-B acute lymphoblastic leukaemia cell line. *Nature*. 1977;267(5614):841-843.
22. Kohno S, Minowada J, Sandberg AA. Chromosome evolution of near-haploid clones in an established human acute lymphoblastic leukemia cell line (NALM-16). *J Natl Cancer Inst*. 1980;64(3):485-493.
23. Findley HW Jr, Cooper MD, Kim TH, Alvarado C, Ragab AH. Two new acute lymphoblastic leukemia cell lines with early B-cell phenotypes. *Blood*. 1982;60(6):1305-1309.
24. Naumovski L, Morgan R, Hecht F, Link MP, Glader BE, Smith SD. Philadelphia chromosome-positive acute lymphoblastic leukemia cell lines without classical breakpoint cluster region rearrangement. *Cancer Res*. 1988;48(10):2876-2879.
25. Greil J, Gramatzki M, Burger R, et al. The acute lymphoblastic leukaemia cell line SEM with t(4;11) chromosomal rearrangement is biphenotypic and responsive to interleukin-7. *Br J Haematol*. 1994;86(2):275-283.
26. Foley GE, Lazarus H, Farber S, Uzman BG, Boone BA, McCarthy RE. Continuous culture of human lymphoblasts from peripheral blood of a child with acute leukemia. *Cancer*. 1965;18:522-529.
27. Huang CC, Hou Y, Woods LK, Moore GE, Minowada J. Cytogenetic study of human lymphoid T-cell lines derived from lymphocytic leukemia. *J Natl Cancer Inst*. 1974;53(3):655-660.
28. Watanabe S, Shimosato Y, Kameya T, et al. Leukemic distribution of a human acute lymphocytic leukemia cell line (Ichikawa strain) in nude mice conditioned with whole-body irradiation. *Cancer Res*. 1978;38(10):3494-3498.
29. Liao Y, Smyth GK, Shi W. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics*. 2014;30(7):923-930.
30. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol*. 2014;15(12):550.
31. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci USA*. 2005;102(43):15545-15550.
32. Chen J, Bardes EE, Aronow BJ, Jegga AG. ToppGene suite for gene list enrichment analysis and candidate gene prioritization. *Nucleic Acids Res*. 2009;37:W305-W311.
33. Hulsen T, de Vlieg J, Alkema W. BioVenn—a web application for the comparison and visualization of biological lists using area-proportional Venn diagrams. *BMC Genomics*. 2008;9:488.

34. Nordlund J, Backlin CL, Wahlberg P, et al. Genome-wide signatures of differential DNA methylation in pediatric acute lymphoblastic leukemia. *Genome Biol*. 2013;14(9):r105.
35. Lindqvist CM, Lundmark A, Nordlund J, et al. Deep targeted sequencing in pediatric acute lymphoblastic leukemia unveils distinct mutational patterns between genetic subtypes and novel relapse-associated genes. *Oncotarget*. 2016;7(39):64071-64088.
36. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods*. 2001;25(4):402-408.
37. Zhao L, Glazov EA, Pattabiraman DR, et al. Integrated genome-wide chromatin occupancy and expression analyses identify key myeloid pro-differentiation transcription factors repressed by Myb. *Nucleic Acids Res*. 2011;39(11):4664-4679.
38. DuVall AS, Sheade J, Anderson D, Yates SJ, Stock W. Updates in the management of relapsed and refractory acute lymphoblastic leukemia: an urgent plea for new treatments is being answered! *JCO Oncol Pract*. 2022;18(7):479-487.
39. Fioretos T, Wirta V, Cavelier L, et al. Implementing precision medicine in a regionally organized healthcare system in Sweden. *Nat Med*. 2022;28(10):1980-1982.
40. Cao L, Mitra P, Gonda TJ. The mechanism of MYB transcriptional regulation by MLL-AF9 oncoprotein. *Sci Rep*. 2019;9(1):20084.
41. Campanero MR, Armstrong M, Flemington E. Distinct cellular factors regulate the c-myb promoter through its E2F element. *Mol Cell Biol*. 1999;19(12):8442-8450.
42. Sanda T, Lawton LN, Barrasa MI, et al. Core transcriptional regulatory circuit controlled by the TAL1 complex in human T cell acute lymphoblastic leukemia. *Cancer Cell*. 2012;22(2):209-221.
43. Mitra P, Pereira LA, Drabsch Y, Ramsay RG, Gonda TJ. Estrogen receptor-alpha recruits P-TEFb to overcome transcriptional pausing in intron 1 of the MYB gene. *Nucleic Acids Res*. 2012;40(13):5988-6000.
44. Pereira LA, Hugo HJ, Malaterre J, et al. MYB elongation is regulated by the nucleic acid binding of NFkappaB p50 to the intronic stem-loop region. *PLoS One*. 2015;10(4):e0122919.
45. Jerchel IS, Hoogkamer AQ, Aries IM, et al. RAS pathway mutations as a predictive biomarker for treatment adaptation in pediatric B-cell precursor acute lymphoblastic leukemia. *Leukemia*. 2018;32(4):931-940.
46. Pasello M, Giudice AM, Scotlandi K. The ABC subfamily a transporters: multifaceted players with incipient potentialities in cancer. *Semin Cancer Biol*. 2020;60:57-71.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Fehr A, Arvidsson G, Nordlund J, Lönnerholm G, Stenman G, Andersson MK. Increased *MYB* alternative promoter usage is associated with relapse in acute lymphoblastic leukemia. *Genes Chromosomes Cancer*. 2023;62(10):597-606. doi:10.1002/gcc.23151