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Original Article

Functional polymorphism in the 3'UTR of IKZF1 with risk of childhood acute lymphoblastic leukemia in a Chinese population

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Abstract: IKZF1 is an essential tumor suppressor that regulates the mechanisms of tumorigenesis and leukemogenesis. IKZF1 polymorphisms have been previously identified with the risk of childhood acute lymphoblastic leukemia (ALL). In this study, we aimed to explore the potential functional polymorphism located in the 3'UTR of IKZF1 to evaluate its relationship with a risk for ALL in Chinese children. In this study, total 570 children diagnosed with ALL and 670 controls were enrolled in the study. The miR-binding SNPs of IKZF1 gene were screened through the loss-or-gain strategy and genotyped using TaqMan allelic discrimination assays. Parental alcohol consumption and house painting status had significantly different distributions among the cases and controls through the questionnaire survey. The genotype distribution revealed the G to A polymorphism (rs11552046) which indicated an increased risk for ALL. This risk was also associated with the percentage of blast cells and total number of white blood cells (WBC). IKZF1 with AA genotype presented a higher binding ability by miR-1976, causing a relatively decreased expression level of IKZF with GA or AA genotype. In conclusion, the rs11552046 in IKZF1 was highly associated with an increased risk of childhood ALL in a Chinese population and might serve as a novel biomarker for the disease.

Keywords: SNP, IKZF1, miR-1976, 3'-UTR, ALL

Introduction

Acute leukemia consists of a group of heterogeneous malignancies in which immature and dysfunctional hematopoietic progenitors proliferate and accumulate in the bone marrow [1, 2]. Acute lymphoblastic leukemia (ALL), the most common cancer among children, is a clonal hematological disease in which undifferentiated or poorly differentiated lymphocytes proliferate indefinitely in hematopoietic tissue [3, 4]. Approximately 85% of all pediatric ALL is B-lineage ALL [5, 6]. In developing countries, including India, cure-rates of children with cancer is inferior (~10-30%) compared to high-income countries (~80%) [7, 8]. In the remaining 15-20% of pediatric ALL patients, relapse is the major cause of death [9]. Most genetic association studies of ALL have focused on candidate genes, primarily those implicated in the metabolism of carcinogens, folate metabolism, immune function, and cell-cycle regulation [10, 11]. Epidemiological studies have assessed the role of environmental factors in the pathogenesis of childhood ALL however these factors alone appear to make a relatively minor contribution to disease risk [12]. Recent genome-wide association studies (GWAS) have identified common genetic variation near IKZF1 (7p12.2), ARID5B (10q21.2), and CEBPE (14q11.2) that influences ALL risk in non-Hispanic white populations [3]. The genes identified from the GWAS play roles in transcriptional regulation and differentiation of B-cell progenitors [13]. The second GWAS study showed that the IKAROS family zinc finger 1 (IKZF1) germline polymorphism rs4132601 is associated with inherited risk of pediatric ALL [14]. Previous study from other laboratories also found that polymorphisms in IKZF1 (7p12.2), ARID5B (10q21.2), and CEBPE (14q11.2), were associated with the risk of childhood ALL [15].

Table 1. Frequency distributions of selected variables in ALL cases and cancer-free controls

Variables	Cases (*n*=570)		Controls (*n*=670)		*P**
	N	%	*N*	%	
Age (years)					
≤6	347	60.9	380	56.7	0.138
>6	223	39.1	290	43.3	
Gender					
Male	349	61.2	440	65.7	0.105
Female	221	38.8	230	34.3	
Parental smoking status					
Negative	216	37.9	290	43.3	0.054
Positive	354	62.1	380	56.7	
Parental drinking status					
Negative	348	61.1	520	77.6	<0.0001
Positive	222	38.9	150	22.4	
House painting status					
Negative	373	65.4	527	78.7	<0.0001
Positive	197	34.6	143	21.3	

*Two-sided chi-square test for analyzing the clinical characteristics of All cases and cancer-free controls.

The product of the IKZF1 gene is Ikaros, a sequence-specific DNA-binding protein with a zinc finger motif, which plays a role in immune homeostasis through the transcriptional regulation of the earliest stages of lymphocyte ontogeny and the differentiation by both gene transcriptional activation and repression [16]. IKZF1 encodes the transcription factor Ikaros, a zinc-finger nuclear protein required for normal lymphoid development [17]. Deletion of IKZF1 results in expression of non-DNA-binding (dominant negative) isoforms of Ikaros, which appear to collaborate with BCR-ABL1 in the pathogenesis of ALL and blastic transformation of CML. IKZF1 deletions have been shown to have a negative prognostic impact in Ph^+ ALL, conferring a shorter disease-free survival even in the era of combined TKI and chemotherapy [18].

MicroRNAs (miRNAs) are small, non-coding RNA molecules that range in 19-25 base pairs in length and regulate gene expression through transcriptional repression of target mRNAs [19]. Previous studies have shown that SNPs located in miRNA binding sites within the 3'UTR may affect the binding affinities between a miRNA and its target mRNA, thus affecting the expression level and function of the target gene [20]. Altered binding between a miRNA and its target may also potentially be associated with various cancers

In this study, we focused on the SNPs in the 3'UTR of IKZF1 by using the bioinformatics software with the loss-or-gain strategy. Based on this, we further investigated the association between the allele distribution and the susceptibility of ALL in a case-control study.

Materials and methods

Study subjects

The study protocol was approved by the local Ethics Committee of Chlidren'S Hospital of Soochow University, and written informed consent was obtained from all participants prior to data collection and the methods were carried out in accordance with the approved guidelines. Subjects ranged in age from 1 to 18 years old. All patients were diagnosed by Morphology, Immunology, Cytogenetics and Molecular Biology (MICM) according to the established guidelines for diagnosis and treatment of childhood ALL in China (Society of Pediatrics, Chinese Medical Association, 2006). Children with other hematological disorders, a previous history of cancer, radiotherapy or chemotherapy were excluded from this study. All cases and controls were age- (±5 years) and gender-matched. A questionnaire was administered in person to study subjects to collect demographic data and environmental exposure information, including the house painting status, parental alcohol use and cigarette use status. Before administering the questionnaire, we obtained written informed consent from parents of suitable subjects. Parental drinking status was considered negative if neither of the parents imbibed alcohol. Parental smoking status was defined as negative if neither parent smoked during or after the pregnancy. The house painting status was considered negative if the house was not painted during the pregnancy or after birth. A total of 1240 subjects, including 570 ALL patients and 670 controls, were recruited to the study (**Table 1**). The total number of white blood cells (WBC) was obtained through the routine blood examination.

SNP selection

Bioinformatics analysis was applied to confirm the function of miRNA associated SNP in the 3'UTR of IKZF1 which could be regulated by different miRNAs. If SNPs were in high linkage disequilibrium (r^2>0.8), we genotyped only one SNP. For the loss-or-gain strategy analysis, we got miRNA wild sequence and SNP allele sequence. Then, we used two target prediction tools (targetscan http://www.targetscan.org/) and miranda (http://www.microrna.org) to predict their target sites respectively. Four categories of results are recorded as wild targescan site (WT), wild miranda site (WM), SNP targetscan site (ST) and SNP miranda site (SM). If one miRNA's target gene shows in both WT and WM, but not in either ST or SM, we called that the miRNA lost this target gene. On the contrary, if one target shows in both ST and SM, but not in either WT or WM, we called that the miRNA gained one target gene.

Genotype

Genomic DNA was extracted from leukocyte pellets by traditional proteinase K digestion, followed by phenol-chloroform extraction and ethanol precipitation. Genotyping was performed with the TaqMan SNP Genotyping Assay. The PCR reactions were carried out in a total volume of 5 μL containing TaqMan Universal Master Mix, 80X SNP Genotyping AssayMix, Dnase-free water and 10-ng of genomic DNA. The PCR conditions were 2 min at 50°C, 10 min at 95°C, followed by 40 cycles at 95°C for 15 sec and 60°C for 1 min in the 384-well ABI 7900HT Real Time PCR System.

Real time PCR assay

Total RNA was obtained from tissues using TRIzol reagent as described by the manufacturer (Invitrogen Life Technologies Co, CA, USA). For mRNA detection, total RNAs (500 ng) were reverse transcribed using the reverse transcription kit (Takara, Tokyo, Japan). GAPDH was used as an internal control. The qRT-PCR was performed using ABI Prism 7900HT (Applied Biosystems, CA, USA) according to the direction of the reagents. The amplification conditions were 95°C for 10 minutes, followed by 40 cycles of 95°C for 30 seconds, 55°C for 40 seconds, and 72°C for 30 seconds, and finally 4°C for 30 minutes for cooling.

Cell lines and cell culture

Jurkat and 6T-CEM cell lines were purchased from the Chinese Academy of Sciences Cell Bank. All cells were cultured in RPMI-1640 (Gibco, USA) supplemented with 10% fetal bovine serum (Invitrogen, Carlsbad, USA) and grown in humidified 5% CO_2 at 37°C. The miR-1976 mimics and normal control were obtained from Genscript (Nanjing, China). The transfection was conducted by using Lipofectamine 3000 (Invitrogen Corp, CA, USA).

Construction of luciferase-based reporter plasmids

The fragments containing the 3'UTR with the G or C alleles of SNP rs11552046 were amplified by Genscript (Nanjing, Jiangsu, China). The PCR production was cloned into the pGL3-promoterless luciferase-based plasmid (Promega, CA, USA) at the cloning site between BamHI and XhoI. The amplified fragment was verified by DNA sequencing with Genscript (Nanjing, Jiangsu, China).

Dual-luciferase reporter assay

The 3'-UTR sequence of IKZF1 predicted to interact with miR-1976 or the mutated sequence with the predicted target sites were inserted into the BamHI and XhoI sites of pGL3 promoter vector (Genscript, Nanjing, China). Jurkat and 6T-CEM cell lines cultured in 24-well plates were co-transfected with pMIR--REPORT vectors containing either the wild-type or mutated IKZF1-3'-UTR segments along with the control vector, Renilla luciferase reporter vector (pRL-TK). These Jurkat and 6T-CEM cells were co-transfected with the precursor microRNAs for miR-1976 and control group. Assays were performed to determine the level of gene expression

Statistical analysis

Statistical analyses were performed using SPSS software version 16.0 (Chicago, IL, USA) and STATA10.0 (San Francisco CA USA). Data normality of distribution was verified before using the Student t test. The results for continuous variables with normal distribution are shown as mean ± standard deviation (SD); otherwise, data are expressed as median and interquartile range. Hardy-Weinberg equilibrium of the geno-

Table 2. Genotype frequencies of the IKZF1 rs11552046 polymorphism among ALL cases and controls

Genotype	Cases (*n*=570)		Controls (*n*=670)		OR (95% CI)	*P* Value
	N	%	*N*	%		
rs11552046						
GG	512	89.8	653	97.5	1.00	
GA	36	6.3	11	1.6	1.11 (1.08-1.44)	
AA	22	3.9	6	0.9	1.95 (1.41-2.39)	<0.001
A carrier	58	10.2	17	2.5	1.78 (1.19-1.96)	<0.001

Table 3. Stratified analysis of rs11552046 genotype with clinicopathological parameters of ALL

Feather	Genotype				GA vs GG	AA vs GG	A carrier vs GG
	GG	GA	AA	A carrier	*P* Value	*P* Value	*P* Value
Age (years)							
≤6	309	21	17	38	0.81	0.11	0.45
>6	203	15	5	20			
Gender							
Male	310	22	17	39	0.95	0.12	0.32
Female	202	14	5	19			
% of Blast cells							
≤10%	345	13	3	16	<0.001	<0.001	<0.001
>10%	167	23	19	42			
WBC (*10^9)							
≤20	309	13	8	21	0.004	0.025	<0.001
>20	203	23	14	37			

*Two-sided chi-square test for either genotype distributions or allele frequencies between cases and controls.

types for each SNP was tested using a Chi-square test in control groups. The association between SNPs and ALL susceptibility was calculated as the odds ratio (OR) and its 95% confidence intervals (CI) in a multivariable logistic regression analysis. All statistical tests were two-sided and P<0.05 was considered statistically significant. The graphs were generated by Graphpad Prism 5.0 (Graphpad Software, Inc.).

Results

Subject characteristics

Differences in the distribution of the selected demographic variables among controls and ALL were presented in **Table 1**. No significant differences in age, sex or parental smoking status were identified between the case and control groups. In the case group, there were more parents positive for alcohol consumption (P< 0.001). In addition, significantly more ALL cases than controls had their house painted during the mother's pregnancy or after the child's birth (P<0.001).

Genotype and allele analysis

The genotypic distribution of the IKZF1 rs-11552046 G>A for cases and controls is presented on **Table 2**. Hardy-Weinberg equilibrium was observed for genotype distribution in the controls. The genotype distribution was statistically significant between the case and control groups (P=0.012). Further, logistic regression analysis results revealed that the GA genotype and AA genotype presented a significantly increased susceptibility of ALL as compared with the GG genotype with the OR=1.11, 95% CI=1.08-1.44; OR=1.95, 95% CI=1.41-2.39, respectively (*P*=0.014). Besides, the A carrier also indicated an increased susceptibility (*P*<0.001), indicating that the GA/AA genotype was associated with an increased risk of childhood ALL.

In a further analysis, we observed an association between the rs11552046 polymorphism and clinicopathological parameters of ALL (**Table 3**). The combined GA/AA genotype was associated with a significantly increased risk for the percentage of blast cells and the total number of WBC in ALL children. No significant association, however, was identified regarding the age or gender.

The miR-1976 could bind with the 3'UTR of IKZF1 with C allele

As predicted by bioinformatics analysis, the potential binding site of miR-1976 in the 3'UTR of IKZF1 was predicted in **Figure 1A**. Using RT-PCR, a significant difference in the IKZF1 expression levels of patients carrying GG, GA and AA genotype was observed (**Figure 1B**). The

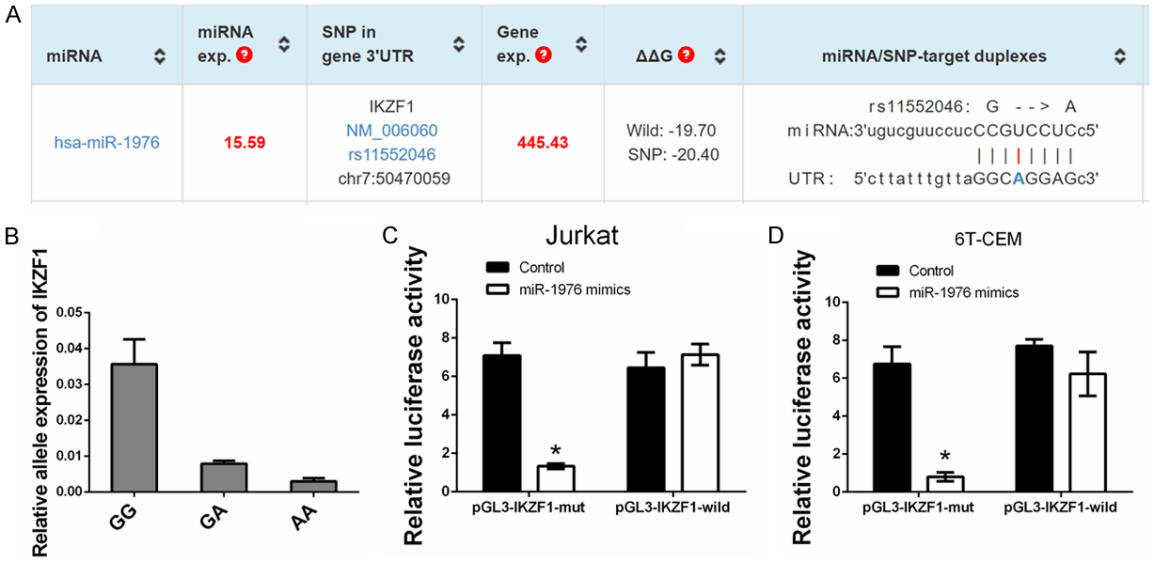

Figure 1. The miR-1976 binding site could be abolished by SNP located in the 3'UTR of IKZF1. A: The potential binding site of miR-1976 in the 3'UTR of IKZF1. B: Relative expression level of IKZF1 was determined by RT-PCR in patients with GG, GA and AA genotypes. C and D: Relative luciferase expression levels obtained from pMIR–REPORT miRNA expression vectors with GG genotype or AA genotype of IKZF1 3'UTR regions following co-transfection with precursors of control or miR-1976, and the Renilla control vector were calculated as the ratio of firefly/renilla luciferase activities in the cells and normalized to those of the control. Data was presented as the mean ± SEM. *indicates a significant difference ($P<0.05$).

expression levels of the GA carriers and the AA carriers were higher than those of GG carriers.

To determine if the rs11552046 polymorphism affected the potential binding of miRNAs to the 3'UTR of IKZF1 mRNA, Jurkat and 6T-CEM cells were transiently transfected with the IKZF1 3'UTR luciferase construct, containing either the wildtype G allele or the mutant A allele. Relative activities were measured with a Dual-Glo Luciferase Reporter Assay System. Cotransfection of the luciferase vector with the 3'UTR of IKZF1 containing the mutated A allele and Hsa-miR-1976 into Jurkat cells significantly altered the luciferase expression level compared with the reporter plasmid containing the wild-type allele. While the mutant allele construct showed reduced luciferase in the presence of miR-1976, luciferase levels in the wild allele construct in the presence of miR-1976 did not appreciably change (**Figure 1C**). The same results were observed in 6T-CEM cells (**Figure 1D**). Thus, we conclude that the rs11552046 polymorphism alters the binding affinity of miR-1976, with the 3'UTR of IKZF1 mRNA.

Discussion

Acute lymphoblastic leukemia is a multifactorial disease influenced by genetic and environmental factors [21]. In this study we investigated whether SNPs which were previously reported in GWAS studies influence the risk of ALL in the Han Chinese population. High CRLF2 mRNA expression and IKZF1 deletions were associated with low survival rates in pediatric BCP-ALL [22]. Among patients with IKZF1 deletion, those with p210 transcripts had significantly shorter DFS than those with p190 transcripts ($p< 0.0001$) [23]. Notably, Chiaretti et al. recently reported worse outcomes among p210 Ph^+ ALL patients treated with a dasatinib-based protocol, associated with a lower susceptibility to TKI, lower blast clearance, and greater incidence of relapse [24]. Novel strategies to target IKZF1 are being examined. In a recently reported high content screening of hundreds of FDA-approved compounds, retinoid receptor agonists were found to reverse the leukemogenic phenotype driven by IKZF1 alterations [25]. IKZF1, which encodes Ikaros, is the founding member of a family of zinc finger transcription factors required for the development of all lymphoid lineages. IKZF1 alterations are present in more than 70% of BCR-ABL1 lymphoid leukemias and have been associated with poor prognoses in BCR-ABL ALL [26]. Evidence from homozygous mutant mice have shown that deletion of IKZF1 leads to a rapid development of leukemia [27].

Here in this study, we identified the rs11552046 polymorphism located in the 3'UTR of IKZF1 and investigated its association with childhood ALL in a Han Chinese population. This polymorphism has never been examined in relationship with diseases. We found that the G to A mutation of rs11552046 significantly increased the risk of ALL. The expression levels of IKZF1 mRNA were significantly lower in the patients carrying either the GA or AA genotype, as compared to patients carrying the GG genotype. Furthermore, a dual-luciferase reporter assay suggested that the A allele significantly affected the binding affinity of miR-1976 to the IKZF1 3'UTR, which would ultimately affect the IKZF1 transcript stability. These data indicate that the rs11552046 polymorphism might serve as a novel biomarker in assessing childhood ALL risk.

Disclosure of conflict of interest

None.

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References

[1] Olshanskaya Y, Kazakova A, Tsaur G, Zerkalenkova E, Soldatkina O, Aprelova E, Plekhanova O, Gindina T, Mercur'ev D, Barhkatov I, Baidun L, Bydanov O, Lagoiko S, Tallen G, Rumiantseva J, Rumiantsev A, Karachunskii A and Henze G. Clinical significance of cytogenetic changes in childhood T-cell acute lymphoblastic leukemia: results of the multicenter group Moscow-Berlin (MB). Leuk Lymphoma 2019; 60: 426-432.

[2] Spencer Noakes TL, Przybycien TS, Forwell A, Nicholls CJ, Zhou YQ, Butcher DT, Weksberg R, Guger SL, Spiegler BJ, Schachar RJ, Hitzler J, Ito S, van der Plas E and Nieman BJ. Brain development and heart function after systemic single-agent chemotherapy in a mouse model of childhood leukemia treatment. Clin Cancer Res 2018; 24: 6040-6052.

[3] Yeoh AEJ, Lu Y, Chin WHN, Chiew EKH, Lim EH, Li Z, Kham SKY, Chan YH, Abdullah WA, Lin HP, Chan LL, Lam JCM, Tan PL, Quah TC, Tan AM and Ariffin H. Intensifying treatment of childhood B-lymphoblastic leukemia with IKZF1 deletion reduces relapse and improves overall survival: results of malaysia-Singapore ALL 2010 study. J Clin Oncol 2018; 36: 2726-2735.

[4] Li Z, He S and Look AT. The MCL1-specific inhibitor S63845 acts synergistically with venetoclax/ABT-199 to induce apoptosis in T-cell acute lymphoblastic leukemia cells. Leukemia 2019; 33: 262-266.

[5] Marz M, Meyer S, Erb U, Georgikou C, Horstmann MA, Hetjens S, Weiss C, Fallier-Becker P, Vandenhaute E, Ishikawa H, Schroten H, Durken M and Karremann M. Pediatric acute lymphoblastic leukemia-Conquering the CNS across the choroid plexus. Leuk Res 2018; 71: 47-54.

[6] Warrick K, Althouse SK, Rahrig A, Rupenthal J and Batra S. Factors associated with a prolonged hospital stay during induction chemotherapy in newly diagnosed high risk pediatric acute lymphoblastic leukemia. Leuk Res 2018; 71: 36-42.

[7] Wen J, Maxwell RR, Wolf AJ, Spira M, Gulinello ME and Cole PD. Methotrexate causes persistent deficits in memory and executive function in a juvenile animal model. Neuropharmacology 2018; 139: 76-84.

[8] Wiemels JL, Walsh KM, de Smith AJ, Metayer C, Gonseth S, Hansen HM, Francis SS, Ojha J, Smirnov I, Barcellos L, Xiao X, Morimoto L, McKean-Cowdin R, Wang R, Yu H, Hoh J, DeWan AT and Ma X. GWAS in childhood acute lymphoblastic leukemia reveals novel genetic associations at chromosomes 17q12 and 8q24.21. Nat Commun 2018; 9: 286.

[9] Sun W, Triche T Jr, Malvar J, Gaynon P, Sposto R, Yang X, Bittencourt H, Place AE, Messinger Y, Fraser C, Dalla-Pozza L, Salhia B, Jones P, Wayne AS, Gore L, Cooper TM and Liang G. A phase 1 study of azacitidine combined with chemotherapy in childhood leukemia: a report from the TACL consortium. Blood 2018; 131: 1145-1148.

[10] Dupuis LL, Johnston DL, Baggott C, Hyslop S, Tomlinson D, Gibson P, Orsey A, Dix D, Price V, Vanan M, Portwine C, Kuczynski S, Spiegler B, Tomlinson GA and Sung L. Validation of the symptom screening in pediatrics tool in children receiving cancer treatments. J Natl Cancer Inst 2018; 110: 661-668.

[11] Roti G, Qi J, Kitara S, Sanchez-Martin M, Saur Conway A, Varca AC, Su A, Wu L, Kung AL, Ferrando AA, Bradner JE and Stegmaier K. Leukemia-specific delivery of mutant NOTCH1 targeted therapy. J Exp Med 2018; 215: 197-216.

[12] Whitehead TP, Metayer C, Wiemels JL, Singer AW and Miller MD. Childhood leukemia and primary prevention. Curr Probl Pediatr Adolesc Health Care 2016; 46: 317-352.

[13] Kuang WY, Zheng MC, Li WL, Yang HX, Zhang BS and Wu P. [Effects of minimal residual dis-

ease level on day 33 of remission induction and IKZF1 genotype on the survival of children with B-lineage acute lymphoblastic leukemia]. Zhongguo Dang Dai Er Ke Za Zhi 2018; 20: 538-542.

[14] Boutboul D, Kuehn HS, Van de Wyngaert Z, Niemela JE, Callebaut I, Stoddard J, Lenoir C, Barlogis V, Farnarier C, Vely F, Yoshida N, Kojima S, Kanegane H, Hoshino A, Hauck F, Lhermitte L, Asnafi V, Roehrs P, Chen S, Verbsky JW, Calvo KR, Husami A, Zhang K, Roberts J, Amrol D, Sleaseman J, Hsu AP, Holland SM, Marsh R, Fischer A, Fleisher TA, Picard C, Latour S and Rosenzweig SD. Dominant-negative IKZF1 mutations cause a T, B, and myeloid cell combined immunodeficiency. J Clin Invest 2018; 128: 3071-3087.

[15] Wang Y, Chen J, Li J, Deng J, Rui Y, Lu Q, Wang M, Tong N, Zhang Z and Fang Y. Association of three polymorphisms in ARID5B, IKZF1 and CEBPE with the risk of childhood acute lymphoblastic leukemia in a Chinese population. Gene 2013; 524: 203-207.

[16] Marke R, van Leeuwen FN and Scheijen B. The many faces of IKZF1 in B-cell precursor acute lymphoblastic leukemia. Haematologica 2018; 103: 565-574.

[17] Sadras T, Heatley SL, Kok CH, Dang P, Galbraith KM, McClure BJ, Muskovic W, Venn NC, Moore S, Osborn M, Revesz T, Moore AS, Hughes TP, Yeung D, Sutton R and White DL. Differential expression of MUC4, GPR110 and IL2RA defines two groups of CRLF2-rearranged acute lymphoblastic leukemia patients with distinct secondary lesions. Cancer Lett 2017; 408: 92-101.

[18] van der Veer A, Zaliova M, Mottadelli F, De Lorenzo P, Te Kronnie G, Harrison CJ, Cave H, Trka J, Saha V, Schrappe M, Pieters R, Biondi A, Valsecchi MG, Stanulla M, den Boer ML and Cazzaniga G. IKZF1 status as a prognostic feature in BCR-ABL1-positive childhood ALL. Blood 2014; 123: 1691-1698.

[19] Tang J, Zhuo H, Zhang X, Jiang R, Ji J, Deng L, Qian X, Zhang F and Sun B. A novel biomarker Linc00974 interacting with KRT19 promotes proliferation and metastasis in hepatocellular carcinoma. Cell Death Dis 2014; 5: e1549.

[20] Liu C, Zhang Y, Chen H, Jiang L and Xiao D. Function analysis of rs9589207 polymorphism in miR-92a in gastric cancer. Tumour Biol 2016; 37: 4439-44.

[21] Bolton-Gillespie E, Schemionek M, Klein HU, Flis S, Hoser G, Lange T, Nieborowska-Skorska M, Maier J, Kerstiens L, Koptyra M, Muller MC, Modi H, Stoklosa T, Seferynska I, Bhatia R, Holyoake TL, Koschmieder S and Skorski T. Genomic instability may originate from imatinib-refractory chronic myeloid leukemia stem cells. Blood 2013; 121: 4175-4183.

[22] Sutton R, Venn NC, Law T, Boer JM, Trahair TN, Ng A, Den Boer ML, Dissanayake A, Giles JE, Dalzell P, Mayoh C, Barbaric D, Revesz T, Alvaro F, Pieters R, Haber M, Norris MD, Schrappe M, Dalla Pozza L and Marshall GM. A risk score including microdeletions improves relapse prediction for standard and medium risk precursor B-cell acute lymphoblastic leukaemia in children. Br J Haematol 2018; 180: 550-562.

[23] Suzuki K, Ono R, Ohishi K, Masuya M, Kataoka I, Liu B, Nakamori Y, Ino K, Monma F, Hamada H, Kitamura T, Katayama N and Nosaka T. IKAROS isoform 6 enhances BCR-ABL1-mediated proliferation of human CD34+ hematopoietic cells on stromal cells. Int J Oncol 2012; 40: 53-62.

[24] Chiaretti S, Vitale A, Vignetti M, Piciocchi A, Fazi P, Elia L, Falini B, Ronco F, Ferrara F, De Fabritiis P, Luppi M, La Nasa G, Tedeschi A, Califano C, Fanin R, Dore F, Mandelli F, Meloni G and Foa R. A sequential approach with imatinib, chemotherapy and transplant for adult Ph+ acute lymphoblastic leukemia: final results of the GIMEMA LAL 0904 study. Haematologica 2016; 101: 1544-1552.

[25] Churchman ML, Low J, Qu C, Paietta EM, Kasper LH, Chang Y, Payne-Turner D, Althoff MJ, Song G, Chen SC, Ma J, Rusch M, McGoldrick D, Edmonson M, Gupta P, Wang YD, Caufield W, Freeman B, Li L, Panetta JC, Baker S, Yang YL, Roberts KG, McCastlain K, Iacobucci I, Peters JL, Centonze VE, Notta F, Dobson SM, Zandi S, Dick JE, Janke L, Peng J, Kodali K, Pagala V, Min J, Mayasundari A, Williams RT, Willman CL, Rowe J, Luger S, Dickins RA, Guy RK, Chen T and Mullighan CG. Efficacy of retinoids in IKZF1-mutated BCR-ABL1 acute lymphoblastic leukemia. Cancer Cell 2015; 28: 343-356.

[26] Hashiguchi J, Onozawa M, Oguri S, Fujisawa S, Tsuji M, Okada K, Nakagawa M, Hashimoto D, Kahata K, Kondo T, Shimizu C and Teshima T. Development of a fluorescence in situ hybridization probe for detecting IKZF1 deletion mutations in patients with acute lymphoblastic leukemia. J Mol Diagn 2018; 20: 446-454.

[27] Bielska M, Borowiec M, Jesionek-Kupnicka D, Braun M, Bojo M, Pastorczak A, Kalinka-Warzocha E, Prochorec-Sobieszek M, Robak T, Warzocha K, Mlynarski W and Lech-Maranda E. Polymorphism in IKZF1 gene affects clinical outcome in diffuse large B-cell lymphoma. Int J Hematol 2017; 106: 794-800.