Impact of promoter polymorphisms in key regulators of the intrinsic apoptosis pathway on the outcome of childhood acute lymphoblastic leukemia

Rocio Sanchez,[1] Janick St-Cyr,[1] Marie-Eve Lalonde,[1] Jasmine Healy,[1] Chantal Richer,[1] Vincent Gagné,[1] Caroline Laverdière,[1,2] Lewis B. Silverman,[3,4] Stephen E. Sallan,[3,4] Donna Neuberg,[5] Jeffery L. Kutok,[6] Ekaterini A. Kritikou,[1] Maja Krajinovic,[1,2] and Daniel Sinnett[1,2]

[1]Division of Hematology-Oncology, Research Center, CHU Sainte-Justine, Montreal, Canada; [2]Department of Pediatrics, Faculty of Medicine, Université de Montréal, Canada; [3]Department of Pediatric Oncology, Dana-Farber Cancer Institute, Boston, MA, USA; [4]Division of Hematology/Oncology, Children's Hospital, Boston, MA, USA; [5]Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Boston, MA, USA; and [6]Department of Pathology, Brigham and Women's Hospital, Boston, MA, USA

Impact of promoter polymorphisms in key regulators of the intrinsic apoptosis pathway in childhood acute lymphoblastic leukemia outcome

Rocio Sanchez[1], Janick St-Cyr[1], Marie-Eve Lalonde[1], Jasmine Healy[1], Chantal Richer[1], Vincent Gagné[1], Ekaterini A. Kritikou[1], Maja Krajinovic[1,2], Daniel Sinnett[1,2]

[1]Division of Hematology-Oncology, CHU Sainte-Justine Research Center, Montreal, Canada; [2]Department of Pediatrics, Faculty of Medicine, Université de Montréal, Montreal, Canada.

Supplemental Data

METHODS

Study subjects

Peripheral blood or bone marrow (samples in remission) was collected from all participants and DNA was extracted as previously described[1]. Outcome data and sufficiently long follow-up (patients diagnosed before July 2005) were available for a subset of 266 patients (clinical and demographic characteristics are presented in Table 1) who were included in the survival analysis. These patients underwent treatment with Dana Farber Cancer Institute (DFCI) ALL Consortium protocols 87-01, 91-01, 95-01 or 00-01[2]. Treatment strategies on DFCI protocols include intensive, multi-agent induction therapy, early intensification with weekly, high-dose asparaginase and frequent pulses of vincristine and corticosteroid during continuation therapy[3, 4]. Considering corticosteroid (CS) treatment, all patients received prednisone during the induction phase (40 mg/m2/day);prednisone or dexamethasone were administered during the intensification and continuation phases as 5-day pulses every 3 weeks, until the completion of therapy. Standard-risk (SR) patients received dexamethasone at a dose of 6 mg/m^2/day or prednisone at a dose of 40 mg/m^2/day and high-risk (HR) patients received doses 3 times higher than those received by SR patients except on protocol 00-01 when HR patients received the same dose as SR patients during the continuation treatment phase. The Institutional Review Boards approved the research protocol and informed consent was obtained from all participating individuals and/or their parents.

Identification of promoter SNPs (pSNPs) in apoptosis genes

Amplimers were designed for direct amplification of five ~500bp overlapping PCR fragments to cover the proximal promoter region (defined as the 2kb region upstream of the transcription start site) of each candidate gene (Supplementary Table 1). Individual PCR products from the genomic DNA of all 40 individuals were directly sequenced on both strands on an ABI 3700 sequencer. We evaluated sequence trace

files using PHRED, PHRAP and CONSED software packages (University of Washington, Seattle WA).

Potential heterozygotes were identified using the POLYPHRED program[5] and were verified by manual

inspection of the individual sequence traces. Allele frequencies were measured for each pSNP and Hardy-

Weinberg equilibrium (HWE) was assessed using the $\chi 2$ goodness of fit test in STATA/IC version 10.1

(StataCorp, College Station, TX). Haplotypes were inferred in the discovery cohort for all sites with minor

allele frequencies >5%, using the PHASE software v. 2.1.1[6].

pSNP genotyping and quality control checks

The selected pSNPs were amplified in three multiplex PCR assays and hybridized to LuminexMicroPlexTM–

xTAG beads for genotyping using allele-specific primer extension (ASPE)[7]. The PCR and TAG-ASPE

primers, as well as amplification and reaction conditions are available upon request. Allele calls were

assessed and compiled using the Automatic Luminex Genotyping software[8]. The average genotype call rate

for the pSNPs tested was 97.0%. HWE was assessed and PedCheck (Version 1.1) was used to identify

genotype incompatibilities using parental data[9]. Inconsistent case-parent trios were sequenced for genotype

validation and those that remained inconsistent were removed from the analysis. Similarly, pSNPs that

deviated from HWE in the control group excluded from the analysis.

Statistical analysis

To maximize power, we used a case-control design to test for associations between apoptosis

genepSNPs/pHaps and disease. Logistic regression was used to compare allele and genotype carriership in

patients and controls and to estimate odds ratios (ORs) and 95% confidence intervals (CIs) for each pSNP

(STATA/IC version 10.1). For genes with multiple markers, we reconstructed haplotypes in the QcALL case-

control cohort using the expectation-maximization algorithm within FAMHAP (Version 16)[10], using parental

data when available. Logistic regression was used to estimate haplotype-specific ORs using the most

common haplotype as reference and a likelihood ratio test implemented in FAMHAP was used to test for

global haplotype association with disease status. Association of pSNP genotypes with ALL outcomes was

assessed by event free survival (EFS) and overall survival (OS). Survival differences, estimated by Kaplan-

Meier analysis for patients with different genotypes, were assessed using a log-rank test (IBM SPSS

Statistics, Version 19.0). Time to event or death was measured as the time between diagnosis and the event of

interest. For censored cases, it represented the time from diagnosis to date last known alive without an

event; for longer time durations all times were truncated at 5 years post-treatment. Analyses stratified by risk

group were also performed. For the genetic variants significantly associated with outcome, the hazard ratio

(HR, with a 95% CI) was estimated by univariable Cox regression analysis(IBM SPSS Statistics, Version

19.0). Cox regression was also used for multivariable analysis including genotype, common prognostic

factors (sex, age, white blood cell count (WBC), ploidy) and treatment protocol, in the model[11]. Similar

analyses were performed in the extended QcALL discovery and DFCI replication cohorts, except that

immunophenotype (B- or T-cell leukemia) was also added to the model. A false discovery rate (FDR)

correction was performed to adjust for multiple comparisonsfor association analysis performed with outcome

data using Q-value software and bootstrap approach as described in Storey et al.[12].

REFERENCES

1.   Baccichet A, Sinnett D. Frequent deletion of chromosome 12p12.3 in children with acute lymphoblastic leukaemia. British journal of haematology. 1997;99(1):107-14.

2.   Moghrabi A, Levy DE, Asselin B, Barr R, Clavell L, Hurwitz C, et al. Results of the Dana-Farber Cancer Institute ALL Consortium Protocol 95-01 for children with acute lymphoblastic leukemia. Blood. 2007;109(3):896-904.

3.   Silverman LB, Stevenson KE, O'Brien JE, Asselin BL, Barr RD, Clavell L, et al. Long-term results of Dana-Farber Cancer Institute ALL Consortium protocols for children with newly diagnosed acute lymphoblastic leukemia (1985-2000). Leukemia. 2010;24(2):320-34.

4.   Vrooman LM, Stevenson KE, Supko JG, O'Brien J, Dahlberg SE, Asselin BL, et al. Postinduction dexamethasone and individualized dosing of Escherichia Coli L-asparaginase each improve outcome of children and adolescents with newly diagnosed acute lymphoblastic leukemia: results from a randomized study--Dana-Farber Cancer Institute ALL Consortium Protocol 00-01. J Clin Oncol. 2013;31(9):1202-10.

5.   Stephens M, Sloan JS, Robertson PD, Scheet P, Nickerson DA. Automating sequence-based detection and genotyping of SNPs from diploid samples. Nat Genet. 2006;38(3):375-81.

6.   Stephens M, Donnelly P. A comparison of bayesian methods for haplotype reconstruction from population genotype data. Am J Hum Genet. 2003;73(5):1162-9.

7.   Koo SH, Ong TC, Chong KT, Lee CG, Chew FT, Lee EJ. Multiplexed genotyping of ABC transporter polymorphisms with the Bioplex suspension array. Biological procedures online. 2007;9:27-42.

8.   Bourgey M, Lariviere M, Richer C, Sinnett D. ALG: automated genotype calling of Luminex assays. PLoS One. 2011;6(5):e19368.

9.   O'Connell JR, Weeks DE. PedCheck: a program for identification of genotype incompatibilities in linkage analysis. Am J Hum Genet. 1998;63(1):259-66.

10.   Becker T, Knapp M. Maximum-likelihood estimation of haplotype frequencies in nuclear families. Genetic epidemiology. 2004;27(1):21-32.

11.   Rousseau J, Gagne V, Labuda M, Beaubois C, Sinnett D, Laverdiere C, et al. ATF5 polymorphisms influence ATF function and response to treatment in children with childhood acute lymphoblastic leukemia. Blood. 2011;118(22):5883-90.

12.   Storey JD, Taylor JE, Siegmund D. Strong control, conservative point estimation and simultaneous conservative consistency of false discovery rates: a unified approach. Journal of the Royal Statistical Society: Series B (Statistical Methodology). 2004;66(1):187-205.

Supplementary Table 1. Summary of the PCR primers used for SNP genotyping

Gene (chromosome), DNA variant	SNP ID	Position	PCR primers	
			Forward	Reverse
BCL2 (18q21)			GGCGGCAGATGAATTACAA	TTTGCACTCGAGCCCTATTA
-1534G>A	rs62098660	60,988,146		
-225G>T	rs2279115	60,986,837		
BCL2A1 (15q25)			GTGTTTCATGTCATGTATGAAAGAG	TCTCAGGATTGCTGCCATAGCTA
-1086C>G	rs16971626	80,264,729		
-800A>G	rs62025493	80,264,443		
-683A>G	rs12912717	80,264,326		
BCL2L1 (20q11)			GAATTGCGAAGCTCAGGAAC	CACCACCCTCCAAAGAAAGA
-741A>C	rs45443696	30,311,396		
-603G>C	rs6121209	30,311,258		
BCL2L10 (15q21)			GCCCCCGGTATATTGTTTTT	GGACAGCCTTGAGTTTCTGC
-1687C>T	rs2414132	52,406,658		
-954A>G	rs12912428	52,405,925		
-692C>T	rs2231287	52,405,663		
-493T>C	rs2231289	52,405,498		
-330A>G	rs2231290	52,405,335		
BCL2L11 (2q13)			CCCCGACTCTTCTTTTTCAA	TCCAACAAACTGCAGACCAG
-1728G>A	rs7577824	111,876,941		
-1550A>C	rs7593638	111,877,104		
-1387C>T	rs56952027	111,877,297		
-1194T>C	rs4848393	111,877,350		
-1140insG	rs11378324	111,878,227		
-274G>A	rs7582030	111,876,941		
BAX (19q13)			TCGCTTGAGTCTGGGAGTTC	CTGAACGTGCGTCCTTCAC
-1916T>G	NA	49,456,297		
-1837A>T	rs11671610	49,456,351		
-1820C>T	rs12983717	49,456,841		
-1766G>A	rs182016718	49,457,938		
-1276C>T	rs148221668	49,456,297		
-179A>G	rs4645878	49,456,351		
BAD (11q13)			TAGTTGCTTGGGCAACGGGAAC	TTCTGTATGGGCACAAGCGTCT

-1685insA	rs3831423	64,053,849		
-993A>C	rs8873314	64,053,157		
-283A>G	rs2510066	64,052,447		
BID (22q.11)			GACTACCCGCTTCCTCCTTATGG	CCATTCTTCGTGCAGAGTTCTC
-1124C>T	rs366542	18,258,382		
-477A>G	rs386333	18,257,735		
BAG1 (9p12)			AGGCCCGCACTTGTTGAC	CCAGCAGGCCACTCCTTA
-1725A>G	rs706123	33,266,445		
-1710A>G	rs706122	33,266,430		
-1023C>A	rs11791605	33,265,743		
-685C>G	rs16919130	33,265,405		
-453C>T	rs77174756	33,265,215		
BAG3 (10q26)			GGAAAGCCTTGCCAATAACA	CCCTGAGTCATCGGCTATAA
-1724A>T	rs61869035	121,409,158		
-856G>A	rs2420641	121,410,026		
-788insC/T	rs35117013	121,410,094		
-787delA	rs34330597	121,410,095		
-786insA/TAA	rs35097693	121,410,096		
-212C>T	rs11199059	121,410,670		
BAG4 (8p11)			GATGAGGGCAAAAGAGCATC	GTTTCCTCGCCATTTATCCA
-1103A>C	rs17435276	38,033,004		
-118G>C	rs2270376	38,033,989		
BIRC4 (Xq25)			CTGGGGTTTCACCGTGTTAG	CAGCCTAGGTGAAGG AAACG
-1352C>G	rs5956578	122,992,696		
-1226T>C	rs5958318	122,992,822		
BIRC5 (17q25)			TGAGGGCAGGCAACTGCTGCGGT	AGTTGTAGTCCTCCCGCCGCGT
-1517A>C	rs7210347	76,208,759		
-1425A>G	rs3764383	76,208,851		
-1223C>T	rs3764382	76,209,053		
-1123A>G	rs3764381	76,209,153		
-1001A>G	rs12449899	76,209,275		
-522C>T	rs8073903	76,209,754		
-503C>G	rs8073069	76,209,773		
-121C>T	rs17878467	76,210,157		

MCL1 (1q21)			TTTCCCATAAAAGGGGAAAGGGGC	ATCACTATTTTGCCCAGGCCGGT
-486T>G	rs9803935	150,552,622		
-256C>G	rs3738485	150,552,392		
-194G>T	rs3738484	150,552,330		
-142ins[a]	rs3831987	150,552,290		
APAF1 (12q23)			GCGGAGCAGTCAAATCCCGCC,	AATGTCTAGATGTCCAGGGTCTC
-528A>G	rs2289315	99,038,549		
-370A>C	rs2289317	99,038,707		
-353ins[b]	rs3217465	99,038,724		
-196T>A	rs76396995	99,038,881		
CASP3 (4q34)			CTCTGGGAAGAAGAGGAGCA	AGTCGAATAGGCGCAAGTGT
-1309G>C	rs1405937	185,571,938		
-1147C>T	rs12507711	185,571,776		
-928C>T	rs12108497	185,571,557		
-494C>T	rs12506750	185,571,123		
CASP6 (4q25)			AATTTCTCCTTGATCCCTGGGC	CAGTTGGTTCTGATTGCGCGCC
-1873C>T	rs7660005	110,626,506		
-1850A>G	rs7682236	110,626,479		
-292C>G	rs5030516	110,624,921		
YWHAQ (2p25)			CCCACCACTTTGATCTGCTT	AAACTTGCCCAAGGTCACT
-515T>C	rs10203320	9,771,620		
-95C>G	rs2091210	9,771,200		
YWHAB (20q13)			CTCCAGGATTCAAATCCAATG	CAGGGAACAAAGACCCTTC
-913C>G	rs3092669	43,513,431		
-730T>G	rs3091409	43,513,614		
-531C>T	rs3092248	43,513,813		
-141C>T	rs6031847	43,514,203		
-134T>C	rs6031848	43,514,210		
-7G>T	rs6031849	43,514,337		
+70insCGC	rs35664412	43,514,413		
ENDOG (9q34)			CTGAGGCCCACAGATCACTT	TTTAAGACGCCGGTATGAGG
-1598C>T	rs3115875	131,579,182		
-1201A>G	rs10988130	131,579,579		
-496T>A	rs2997923	131,580,311		

-378A>G	rs12380423	131,580,402		
-293G>A	rs77144778	131,580,487		
-210T>C	rs2977998	131,580,570		
ADPRT (1q41)			CCGTTCCCTGATAGATTGCT	GAGTGGCCATTTAGGGATGA
-1491A>G	rs2793378	226,597,341		
-886T>A	rs2793379	226,596,686		
-589A>G	rs1341336	226,596,389		
-279C>T	rs7527192	226,596,079		
-239C>T	rs2077197	226,596,039		
-219A>T	rs7531668	226,596,019		

DNA variants were numbered with respect to the first nucleotide of the first exon as +1, and the nucleotide immediately upstream as -1 (based on Ref Seq mRNA), SNP position relative to the UCSC Genome Browser Human Feb. 2009 Assembly (GRCh37/hg19). NA, not available i.e. unknown in dbSNP build 135.

[a] 18 bp deletion/insertion variation in the promoter region of MCL1, -142-/GGGGCCTGAGCCGGGGCC

[b] 12 bp deletion/insertion variation in the promoter region of APAF1, -353-/AGCGCCTTCCAC

Supplementary Table 2. Results of the gene reporter assays for all promoter haplotypes tested

Gene	Haplotype studied	Ratio of highest allele activity on pGL3-basic			Ratio of highest allele activity on lowest allele activity		
		HeLa	Jeg-3	HepG2	HeLa	Jeg-3	HepG2
BCL2	H1: G-G	2.2	9.1	2.76			
	H2: G-T	1.3	7.4	1.8			
	H3: A-T	3	9.5	3.2	H3/H2 2.3***	H3/H2 1.2*	H3/H2 1.7***
BCL2A1	H1: G-G-G						
	H2: G-G-A	NA	NA	NA			
	H3: C-A-G						
BCL2L1	H1: A-G	41.2	33.7	27.7	H1/H2 1.5*	H1/H2 1.7***	H1/H2 2.2***
	H2: C-C	26.9	15	16.3			
BCL2L10	H1: C-A-C-T-A						
	H2: C-G-C-T-A	NA	NA	NA			
	H3: T-A-C-C-G						
	H4: T-A-T-T-A						
BCL2L11	H1: A-C-T-C-ins-G	22	45.8	22.6			
	H2: A-C-T-C-ins-A	24.3	50.6	21.2			
	H3: G-A-C-T-del-G	27.5	51.1	21.6			
BAX	H1: T-A-G-G	27.7		21			
	H2: G-T-A-A	24.3		21.2			
BAD	H1: insA-A-G	1.8	1.6				
	H2: insA-C-A	4.36	4.83	NA		H2/H1 2.9***	
	H3: delA-A-G	5.72	4.58		H3/H1 3.1***		
BID	H1: G-T	4	2.86	1.85			
	H2: G-C	7.09	4.22	2.12			
	H3: A-C	7.09	4.45	2.61	H3/H1 1.8***	H3/H1 1.5***	
BAG1	H1: G-A-C-C-C	64	92.6				
	H2: G-A-A-G-C	64.7	70.3	NA			
	H3: G-G-C-C-C	50.2	89.2				
BAG3	H1: A-G-T-A-A-C	28.5	81.6				
	H2: A-A-del-del-del-T	36.5	101.6	NA			
	H3: T-G-T-A-A-C	35.7	108				

BAG4	H1: A-G	96	53.2	46			
	H2: C-C	83	56	40.5			
BIRC4	H1: C-T	20.8	89.4	33.9	H1/H2 1.8**	H1/H2 2.8***	H1/H2 1.2*
	H2: G-T	11.8	31.3	27.2			
	H3: G-C	15.2	60.2	30			
BIRC5	H1: C-A-T-G-A-T-G-C	1.4	NA	NA			
	H2: A-G-T-G-G-T-G-C	1.6					
	H3: C-A-C-A-G-C-C-C	1.7					
	H4: C-A-T-G-G-C-G-T	1.7					
MCL1	H1: G-G-T-del	27.4	67.6			H1/H2 1.8**	
	H2: T-C-G-del	30.4	37.2	NA	H2/H3 1.4***		
	H3: T-C-G-ins	21.7	40.7				
APAF1	H1: G-A-del-T	4.84	20.7				
	H2: G-A-ins-T	15	51	NA	H2/H1 3.1***		
	H3: G-C-del-A	12.5	102			H3/H1 4.9***	
CASPASE 3	H1: G-C-C-C						
	H2: G-T-T-T	NA	NA	NA			
	H3: C-C-T-C						
CASPASE 6	H1: C-A-C	2.8	2.6	3.9			
	H3: T-G-C	4.6	3.3	3.1			
YWHAQ	H1: T-C	182	58		H1/H3 1.4***	H1/H2 1.5***	
	H2: T-G	134	37.8	NA			
	H3: C-G	127	40.6				
YWHAB	H1: G-G-C-C-T-G-del	69.7	595				
	H2: C-T-C-C-T-G-del	93	1416	NA	H2/H1 1.3***	H2/H1 2.3***	
	H3: C-T-C-T-C-T-ins	78.7	933				
ENDOG	H1: T-A-A-G-G-C	13.7	14.6	13	H1/H3 1.8**	H1/H3 1.6***	H1/H3 1.3**
	H2: C-G-T-A-A-T	11.1	11.9	14.6			
	H3: C-G-T-A-G-T	7.4	8.7	9.8			
ADPRT	H1: A-T-A-C-C-A	17.5	4.25	7.1			
	H2: G-T-G-T-T-T	23.4	9.68	13.3			H2/H1 1.8***
	H3: G-A-G-C-C-A	29.2	10.4	12.6	H3/H1 1.7***	H3/H1 2.4***	

NA, not available. * *P* <0.05   ** *P* <0.005   *** *P* <0.0005

Supplementary Table 3. Allele frequencies of apoptosis promoter SNPs in childhood pre-B ALL patients and controls

Gene, DNA variants	Alleles	No.		OR (95% CI)	*P*
		ALL patients	Controls		
ADPRT					
-589A>G	A	412 (67)	442 (68)	1.03 (.82-1.31)	.79
	G	202 (33)	210 (32)		
-219A>T	A	499 (81)	543 (84)	1.19 (.89-1.59)	.24
	T	115 (19)	105 (16)		
APAF1					
-528G>A	G	599 (98)	643 (98)	1.07 (.47-2.44)	.87
	A	11 (2)	11 (2)		
-370A>C	A	547 (90)	597 (92)	1.35 (.92-1.98)	.13
	C	63 (10)	51 (8)		
-196T>A	T	547 (90)	598 (92)	1.32 (.90-1.94)	.15
	A	63 (10)	52 (8)		
BAD					
-1685delA	A	519 (84)	558 (86)	1.11 (.82-1.52)	.51
	-	97 (16)	94 (14)		
-283G>A	G	404 (67)	413 (64)	0.87 (.69-1.10)	.25
	A	202 (33)	237 (36)		
BCL2					
-1534G>A	G	370 (60)	394 (61)	1.02 (.82-1.28)	.84
	A	246 (40)	256 (39)		
-225G>T	G	289 (49)	325 (52)	1.09 (.87-1.36)	.46
	T	295 (51)	305 (48)		
BID					
-1124T>C	T	306 (50)	329 (51)	1.03 (.83-1.29)	.78
	C	308 (50)	321 (49)		
-477G>A	G	484 (80)	529 (83)	1.20 (.90-1.60)	.21
	A	120 (20)	109 (17)		
BIRC4[a]					
Males					
-1352G>C	G	183 (52)	226 (65)	**1.68 (1.24-2.28)**	**.0007**
	C	169 (48)	124 (35)		
Females					
-1352G>C	G	154 (62)	176 (62)	1.00 (.70-1.41)	.99
	C	96 (38)	110 (38)		
MCL1					
-486G>T	G	325 (53)	364 (56)	1.14 (.91-1.42)	.25

	T	289 (47)	284 (44)		
-256G>C	G	323 (54)	364 (57)	1.14 (.91-1.42)	.26
	C	273 (46)	270 (43)		
-94T>G	T	325 (53)	372 (57)	1.16 (.93-1.45)	.18
	G	287 (47)	282 (43)		
YWHAB					
-913C>G	C	373 (64)	410 (64)	0.98 (.78-1.24)	.88
	G	209 (36)	234 (36)		
YWHAQ					
-515T>C	T	427 (69)	448 (69)	1.00 (.79-1.27)	.99
	C	191 (31)	200 (31)		
-95C>G	C	402 (65)	414 (64)	0.93 (.74-1.18)	.56
	G	212 (35)	234 (36)		

[a] *BIRC4* lies within the X chromosome therefore ORs were measured separately in males and females.
Percentages indicate number of chromosomes with given allele/total number of chromosomes in the dataset.
OR indicates crude odds ratio; CI, confidence interval. Significant results are shown in bold.

Supplementary Table 4. Genotype frequencies of apoptosis promoter SNPs in childhood pre-B ALL patients and controls

Gene, DNA variants	Genotype	No. (%)		OR (95% CI)	*P*
		ALL patients	**Controls**		
ADPRT					
-589A>G	AA	136 (44)	149 (46)	1 (referent)	—
	AG	140 (46)	144 (44)	1.07 (.77-1.48)	.71
	GG	31 (10)	33 (10)	1.03 (.60-1.77)	.92
-219A>T	AA	204 (66)	229 (71)	1 (referent)	—
	AT	91 (30)	85 (26)	1.20 (.85-1.71)	.30
	TT	12 (4)	10 (3)	1.35 (.57-3.18)	.50
APAF1					
-528G>A	GG	294 (96)	316 (97)	1 (referent)	—
	GA	11 (4)	11 (3)	1.07 (.46-2.52)	.87
	AA	0	0	—	—
-370A>C	AA	247 (81)	273 (84)	1 (referent)	—
	AC	53 (17)	51 (16)	1.15 (.75-1.75)	.52
	CC	5 (2)	0	—	—
-196T>A	TT	248 (81)	273 (84)	1 (referent)	—
	TA	51 (17)	52 (16)	1.08 (.71-1.65)	.72
	AA	6 (2)	0	—	—
BAD					
-1685DEL A	AA	222 (72)	240 (74)	1 (referent)	—
	A-	75 (24)	78 (24)	1.04 (.72-1.50)	.84
	--	11 (4)	8 (2)	1.49 (.59-3.76)	.40
-283G>A	GG	141 (47)	130 (40)	1 (referent)	—
	GA	122 (40)	153 (47)	0.74 (.52-1.04)	.07
	AA	40 (13)	42 (13)	0.88 (.52-1.48)	.61
BCL2					
-1534G>A	GG	112 (36)	126 (39)	1 (referent)	—
	GA	146 (47)	142 (44)	1.16 (.82-1.63)	.41
	AA	50 (16)	57 (17)	0.99 (.62-1.56)	.96
-225G>T	GG	71 (24)	90 (29)	1 (referent)	—
	GT	147 (50)	145 (46)	1.29 (.87-1.89)	.20
	TT	74 (25)	80 (25)	1.17 (.75-1.83)	.48
BID					
-1124T>C	TT	71 (23)	81 (25)	1 (referent)	—
	TC	164 (53)	167 (51)	1.12 (.76-1.65)	.56
	CC	72 (23)	77 (24)	1.07 (.68-1.68)	.78
-477G>A	GG	196 (65)	220 (69)	1 (referent)	—

	GA	92 (30)	89 (28)	1.16 (.82-1.65)	.40
	AA	14 (5)	10 (3)	1.57 (.68-3.62)	.29
BIRC4[a]					
Males					
-1352G>C	GG	90 (52)	113 (65)	1 (referent)	—
	GC	0	0	—	—
	CC	83 (48)	62 (35)	**1.68 (1.09-2.58)**	**.02**
Females					
-1352G>C	GG	50 (40)	56 (39)	1 (referent)	—
	GC	54 (43)	64 (45)	0.95 (.56-1.60)	.83
	CC	21 (17)	23 (16)	1.02 (.51-2.07)	.95
MCL1					
-486G>T	GG	85 (28)	97 (30)	1 (referent)	—
	GT	155 (50)	170 (52)	1.04 (.72-1.50)	.83
	TT	67 (22)	57 (18)	1.34 (.85-2.12)	.21
-256G>C	GG	85 (29)	102 (32)	1 (referent)	—
	GC	153 (51)	160 (50)	1.15 (.80-1.65)	.46
	CC	60 (20)	55 (18)	1.31 (.82-2.09)	.26
-94T>G	TT	84 (27)	102 (31)	1 (referent)	—
	TG	168 (54)	168 (51)	1.13 (.79-1.63)	.49
	GG	57 (19)	57 (18)	1.38 (.88-2.19)	.16
YWHAB					
-913C>G	CC	121 (42)	124 (39)	1 (referent)	—
	CG	131 (45)	162 (50)	0.83 (.59-1.16)	.28
	GG	39 (13)	36 (11)	1.11 (.66-1.86)	.69
YWHAQ					
-515T>C	TT	151 (49)	157 (49)	1 (referent)	—
	TC	125 (40)	134 (41)	0.97 (.70-1.35)	.86
	CC	33 (11)	33 (10)	1.04 (.61-1.77)	.89
-95C>G	CC	135 (44)	132 (41)	1 (referent)	—
	CG	132 (43)	150 (46)	0.86 (.62-1.20)	.38
	GG	40 (13)	42 (13)	0.93 (.57-1.53)	.78

[a] *BIRC4* lies within the X chromosome therefore ORs were measured separately in males and females. Percentages indicate number of individuals with a given genotype/total number of individuals genotyped at that position in the dataset. OR indicates crude odds ratio; CI, confidence interval. Significant results are shown in bold.

Supplementary Table 5. Distribution of apoptosis promoter haplotypes in childhood pre-B ALL and controls

Haplotype	DNA variant		No. (%)		OR (95% CI)	P	Global X^2 (df)	Global P
	-589A>G	-219A>T	ALL patients	Controls				
ADPRT*1	A	A	420 (67.3)	442 (67.8)	1 (referent)	—		
ADPRT*2	G	A	88 (14.1)	103 (15.8)	0.90 (.65-1.25)	.51	0.62 (2)	.73
ADPRT*3	G	T	116 (18.6)	107 (16.4)	1.14 (.84-1.55)	.38		

Haplotype	DNA variant			No. (%)		OR (95% CI)	P	Global X^2 (df)	Global P
	-528A>G	-370A>G	-196T>A	ALL patients	Controls				
APAF1*1	G	A	T	559 (89.6)	601 (91.9)	1 (referent)	—		
APAF1*2	G	C	A	52 (8.3)	41 (6.3)	1.36 (.87-2.14)	.15	5.45 (4)	.24
APAF1*3	A	C	A	11 (1.8)	11 (1.7)	1.08 (.42-2.76)	.87		
APAF1*	*	*	*	2 (0.3)	1 (0.2)	2.15 (.11-127.0)	0.52		

Haplotype	DNA variant		No. (%)		OR (95% CI)	P	Global X^2 (df)	Global P
	-1685DEL A	-283G>A	ALL patients	Controls				
BAD*1	A	G	204 (32.7)	237 (36.2)	1 (referent)	—		
BAD*2	A	A	320 (51.3)	323 (49.4)	1.15 (.90-1.48)	.26	1.79 (2)	.41
BAD*3	-	G	100 (16.0)	94 (14.4)	1.24 (.87-1.76)	.22		

Haplotype	DNA variant		No. (%)		OR (95% CI)	P	Global X^2 (df)	Global P
	-1534G>A	-225G>T	ALL patients	Controls				
BCL2*1	G	G	305 (48.9)	335 (51.5)	1 (referent)	—		
BCL2*2	A	T	249 (39.9)	256 (39.4)	1.07 (.84-1.36)	.58	2.68 (3)	.44
BCL2*3	G	T	69 (11.1)	59 (9.2)	1.28 (.86-1.92)	.20		
BCL2*	*	*	1 (0.2)	0	—	—		

Haplotype	DNA variant		No. (%)		OR (95% CI)	P	Global X^2 (df)	Global P
	-1124T>C	-477G>A	ALL patients	Controls				
BID*1	T	G	311 (49.8)	329 (50.5)	1 (referent)	—		
BID*2	C	G	186 (29.8)	214 (32.8)	0.92 (.71-1.19)	.51	5.25 (3)	.16
BID*3	C	A	127 (20.4)	107 (16.4)	1.26 (.92-1.72)	.14		
BID*	*	*	0	2 (0.3)	—	—		

Haplotype	DNA variant			No. (%)		OR (95% CI)	P	Global X^2 (df)	Global P
	-486G>T	-256C>G	-1□4G>T	ALL patients	Controls				
MCL1*1	G	G	T	325 (52.1)	368 (56.3)	1 (referent)	—		
MCL1*2	T	C	G	291 (46.6)	278 (42.5)	1.19 (.64-1.49)	.13	16.75 (7)	.02
MCL1*	*	*	*	8 (1.3)	8 (1.2)	□□13 (.37-3.50)	0□81		

Haplotype	DNA variant		No. (%)		OR (95% CI)	P	Global X^2 (df)	Global P
	-515T>C	-95C>G	ALL patients	Controls				
YWHAQ*1	T	C	381 (61.3)	392 (60.3)	1 (referent)	—		
YWHAQ*2	C	G	161 (25.9)	178 (27.4)	0.93 (.71-1.2	□58	2.50 (2)	.48
YWHAQ*3		G	50 (8.0)	57□(8.8)	0.90 (.59-1.38)	.62		
YWHAQ*4	C	C	30 (4.8)	23 (3.5)	1.34 (.74-2.47)	.30		

The risk of ALL was evaluated for each haplotype compared with all other possible haplotypes combined. Percentages indicate number of chromosomes with given haplotype/total number of chromosomes. Haplotypes with relative frequencies <5% are grouped under GENENAME* and are represented as * combinations of the associated DNA variants. A likelihood ratio test was performed in FAMHAP to compare global haplotype differences between cases and controls and is reported here as a Global X^2 test with number of haplotype parameters different from zero-1 degrees of freedom.
OR indicates crude odds ratio; CI, confidence interval; df, degrees of freedom; and —, not applicable.