

Table S1. DNA methylation of 34 candidate regions in JMML and healthy controls.

Gene	Gene selection criteria	Region of analysis, position relative to transcription start site	Number of CpG units analyzed	Methylation range, healthy controls [%]	Threshold hypermethylation [%]*	Methylation range, JMML [%]	Hypermethylated JMML cases
<i>CREBBP</i>	Ref. 8	-1705 to -1269 (variant 1) [#]	13	31.3 – 43.8	48.2	34.7 – 79.5	34 / 44 (77%)
<i>MPO</i>	Ref. 8	+1422 to +1816	10	6.2 – 15.3	20.2	6.0 – 66.0	20 / 45 (44%)
<i>SLC12A8</i>	Ref. 8	+131 to +471 (variant 1) [#]	10	3.4 – 6.1	7.1	2.8 – 48.5	19 / 45 (42%)
<i>HIC2</i>	Ref. 8	-821 to -389	15	5.1 – 7.7	8.6	3.8 – 15.6	13 / 44 (30%)
<i>TLX3</i>	Ref. 8	-71 to 324	13	4.8 – 7.3	8.3	3.8 – 21.4	11 / 45 (24%)
<i>TAL1</i>	Ref. 8	+887 to +1360 (variant 1) [#]	10	4.3 – 5.3	5.8	3.7 – 7.7	8 / 44 (18%)
<i>RINT1</i>	7q22	-90 to +328	11	3.1 – 4.1	4.3	2.7 – 6.7	8 / 45 (18%)
<i>TCF4</i>	Ref. 8	-1540 to -1171 (variant 1) [#]	11	2.9 – 4.9	5.7	2.5 – 16.6	8 / 45 (18%)
<i>LHFPL3</i>	7q22	-28 to +403	9	2.1 – 4.0	4.4	2.2 – 8.4	8 / 45 (18%)
<i>SLC26A5</i>	7q22	+468 to +760 (variant a) [#]	8	3.5 – 6.4	8.1	2.0 – 15.6	7 / 45 (16%)
<i>ESR1</i>	Ref. 8	+224 to +634 (variant 1) [#]	9	3.6 – 5.8	6.8	3.9 – 41.7	6 / 45 (13%)
<i>HIC1</i>	Ref. 8	-3558 to -3235 (variant 1) [#]	4	8.5 – 18.8	22.2	6.0 – 28.8	4 / 45 (9%)
<i>ARMC10</i>	7q22	-163 to +228 (variant a) [#]	9	1.3 – 2.1	2.5	0.9 – 2.7	3 / 42 (7%)
<i>NAPEPLD</i>	7q22	-23 to +290 (variant 1) [#]	7	0.7 – 2.1	2.8	0.7 – 7.6	3 / 45 (7%)
<i>KMT2E (MLL5)</i>	7q22	-942 to -514 (variant 1) [#]	10	1.1 – 2.5	3.1	1.3 – 3.5	2 / 44 (5%)
<i>FAM49B</i>	Ref. 8	-651 to -233 (variant 1) [#]	6	1.5 – 15.0	15.1	1.5 – 15.5	2 / 44 (5%)
<i>PUS7</i>	7q22	+249 to +662 (variant 2/3) [#]	10	3.2 – 6.1	6.9	2.8 – 9.7	2 / 44 (5%)

<i>EPOR</i>	Ref. 8	+539 to +945 (variant 1) [#]	12	2.0 – 4.8	5.3	1.6 – 7.5	2 / 45 (4%)
<i>CXCR4</i>	Ref. 8	-1561 to -1229 (variant 1) [#]	9	2.2 – 3.4	3.7	1.9 – 4.4	2 / 45 (4%)
<i>ETV6</i>	Ref. 8	-964 to -695	14	2.5 – 4.2	4.9	2.3 – 8.4	2 / 45 (4%)
<i>NFAT5</i>	Ref. 8	+447 to +867 (variant 1) [#]	12	1.7 – 3.6	4.2	1.2 – 6.6	2 / 45 (4%)
<i>PMPCB</i>	7q22	-52 to +998	6	1.0 – 3.0	3.8	1.2 – 4.0	1 / 45 (2%)
<i>NUP133</i>	Ref. 8	-280 to +103	11	1.1 – 2.9	3.3	1.3 – 3.7	1 / 45 (2%)
<i>RB1</i>	Ref. 8	+247 to +481	8	1.1 – 2.1	2.5	1.1 – 2.6	1 / 45 (2%)
<i>ZNF25</i>	Ref. 8	-373 to -64	9	1.8 – 3.3	3.7	1.2 – 4.1	1 / 45 (2%)
<i>RUNX1</i>	Ref. 8	-385 to +10 (variant 2/3) [#]	11	1.8 – 3.0	3.5	1.7 – 3.5	0 / 45 (0%)
<i>DAPK1</i>	Ref. 8	-107 to +136 (variant 1) [#]	6	2.5 – 4.0	4.6	2.0 – 3.5	0 / 45 (0%)
<i>SLC25A13</i>	Ref. 8	-19 to +368 (variant 1) [#]	9	2.1 – 4.1	5.4	1.8 – 4.4	0 / 45 (0%)
<i>PRG2</i>	Ref. 8	-36546 to -36169 (variant 1) [#]	12	1.0 – 2.3	2.3	0.8 – 2.2	0 / 45 (0%)
<i>FOS</i>	Ref. 8	-1535 to -1210	13	2.2 – 4.0	4.6	2.5 – 4.5	0 / 45 (0%)
<i>RELN</i>	7q22	-845 to -458 (variant 1) [#]	8	1.1 – 3.4	3.9	0.9 – 3.0	0 / 45 (0%)
<i>KMT2C (MLL3)</i>	Ref. 8	-28349 to -28061	10	2.5 – 4.9	5.7	2.4 – 5.7	0 / 45 (0%)
<i>CDCA5</i>	Ref. 8	-154 to +290	12	2.6 – 4.9	5.6	2.6 – 5.1	0 / 45 (0%)
<i>PBX1</i>	Ref. 8	+16763 to +17233 (variant 1) [#]	16	86.3 – 93.1	96.4	40.3 – 94.6	0 / 45 (0%)

* Three standard deviations above the mean of 11 healthy controls

[#] Transcript variants according to NCBI RefSeq Gene, GRCh37/hg19

Table S2. *CREBBP* sequence variants in 64 cases of JMML.

Patient ID	Ras pathway category	<i>CREBBP</i> NM_004380	<i>CREBBP</i> NM_001079846	Variant allele frequency in this sample	Germline	Estimated population frequency			dbSNP138
						Exome Variant Server	ExAc Browser	1000 Genomes	
D124	PTPN11	exon16:c.C3238G:p.P1080A	exon15:c.C3124G:p.P1042A	0.41	Unknown	0.0001	-	-	rs373586649
D448	Quadruple-negative	exon19:c.A3611T:p.Y1204F	exon18:c.A3497T:p.Y1166F	0.45	Unknown	0.0001	0.0001	-	rs200346970
D561	NF1	exon31:c.A5933G:p.N1978S	exon30:c.A5819G:p.N1940S	0.45	Unknown	0.0043	0.0088	0.002	rs112906840
D567	PTPN11	exon14:c.A2728G:p.T910A	exon13:c.A2614G:p.T872A	0.49	Unknown	0.0023	0.0023	0.000	rs143247685
D598	KRAS	exon07:c.C1651A:p.L551I	exon6:c.C1537A:p.L513I	0.36	Unknown	0.0087	0.0100	0.006	rs61753381
D763	PTPN11	exon07:c.C1651A:p.L551I	exon6:c.C1537A:p.L513I	0.40	Yes	0.0087	0.0100	0.006	rs61753381
D766	PTPN11	exon15:c.G2941A:p.A981T	exon14:c.G2827A:p.A943T	0.34	Yes	0.0041	0.0039	0.002	rs61753380
D823	Quadruple-negative	exon02:c.A493G:p.S165G	exon2:c.A493G:p.S165G	0.59	Yes	-	-	-	-
D854	NRAS	exon31:c.C6449T:p.P2150L	exon30:c.C6335T:p.P2112L	0.43	Yes	-	<0.0001	-	-
D903	NF1	exon14:c.C2678T:p.S893L	exon13:c.C2564T:p.S855L	0.35	Yes	0.0014	0.0008	<0.001	rs142047649
D953	PTPN11	exon16:c.C3230T:p.P1077L	exon15:c.C3116T:p.P1039L	0.46	Unknown	-	0.0082	-	-

Table S3. Correlation of *CREBBP* CpG #8–#10 methylation with clinical and hematologic characteristics of JMML patients.

	N	Correlation with <i>CREBBP</i> methylation		Category	N	<i>CREBBP</i> methylation [%]	
		Spearman	p			Median	p
Total cohort	44				44	18.5	
Age [years]	44	0.471	<0.01				
				<2 years	28	13.8	
				≥ 2 years	16	32.0	<0.01
Sex	44						
				Male	29	14	
				Female	15	29	n.s.
Leukocytes [10 ⁹ /L]	44	0.077	n.s.				
				<30	22	14.3	
				≥30	22	19.0	n.s.
Platelets [10 ⁹ /L]	43	0.074	n.s.				
				Transfused before diagnosis	1		
				<50	11	14.0	
				50-100	11	18.0	
				≥100	21	21.0	n.s.
Hemoglobin [g/dL]	41	-0.056	n.s.				
				Transfused before diagnosis	3		
				<10	27	18.0	
				≥10	14	21.0	n.s.
Myeloblasts (PB) [%]	44	0.220	n.s.				
				<2	20	14.0	
				≥2	24	24.3	n.s.
Myeloblasts (BM) [%]	43	0.180	n.s.				
				Missing	1		
				<5	16	17.3	
				≥5	27	19.0	n.s.
Monocytes (PB) [%]	44	0.110	n.s.				
				<10	6	21.3	
				10-19	15	18.0	
				≥20	23	19.0	n.s.
Monocytes (BM) [%]	43	0.226	n.s.				
				Missing	1		
				<5	14	16.5	
				≥5	29	19.0	n.s.
Spleen size [cm below the costal margin]	42	-0.115	n.s.				
				Missing	2		
				<5cm	27	19.0	
				≥5cm	15	14.5	n.s.
Hemoglobin F (age-adjusted)							
				Normal	12	12.3	
				Elevated	13	32.0	<0.01
				Missing	19		
Karyotype							
				Normal	26	14.0	
				Aberrant	15	27.5	n.s.
				Missing	3		
Mutation							
				<i>NF1</i>	5	32.5	
				<i>PTPN11</i>	15	29.0	
				<i>KRAS</i>	6	13.8	
				<i>NRAS</i>	5	13.5	
				<i>CBL</i>	8	9.3	<0.01
				No mutation	2		
				Missing	3		

Abbreviations: n.s., not significant; PB, peripheral blood; BM, bone marrow

Nonparametric statistics were used to test *CREBBP* methylation for differences between 2 subgroups (Mann-Whitney test) or more than 2 subgroups (Kruskal-Wallis test). P values below or equal to 0.05 were considered to be statistically significant.

Table S4. DNA methylation analysis using mass spectrometry (EpiTYPER, Agena Bioscience).

Target	Forward primer	Reverse primer
<i>ARMC10</i>	TTATTTGATTTGGATGTGGAGAAAG	TTCTCCTTAAAATAATTCTTCAACCTC
<i>CDCA5</i>	GGGAGGGAAGAGGTTATTTTTTAT	ACCACTCTCCCCAAAACCTCTAC
<i>CREBBP</i>	GATATTAGGGAGTGAGGGGGTT	AAAAACAATCTCCCAAATAAAAAAC
<i>CXCR4</i>	TTGTGTTGGGAGATTGGTATAGTTT	TACATATATCTCCCCCTTAAATCCC
<i>DAPK1</i>	AGTTTAGTAATGTGTTATAGGTG	ACCAATAAAAAACCCTACAAAC
<i>EPOR</i>	GGTTGATTTGGTGTAAAGGTTTTT	CCCCTAATTCCCCAAAACAAA
<i>ESR1</i>	TTTTTTATATTAAAGTATTTGGGATGG	TTCTCCAAATAATAAAACACCTACTAACC
<i>ETV6</i>	AAGAGAATTTATTAGGAAATGGGAGA	TTTAATAACTACCCTACAACCTTCCC
<i>FAM49B</i>	GGTTGGGGTTTTTTAGTTTTTTAG	ACCCACTAAATATTTTTACCCAAAATC
<i>FOS</i>	GGAGGTAAGGTGTTTTAGAGTGTGT	TTCCCTATTACTATCTATAACAAAATCTCC
<i>HIC1</i>	GTTGTTTATTATTTTTGGGGAGGTT	CCCTCCTCAATTCCTAAACCTAA
<i>HIC2</i>	GTTTTTGGGAAGGTTATTTGTATGG	AATAAACTTACCCCCTTTTAACCCT
<i>KMT2C (MLL3)</i>	GGTTTTTTAGTGGTTAGGGAGAGAG	AAAACCTAACACCTATCAAATCCACTTT
<i>KMT2E (MLL5)</i>	GTTTATTAGGGGTTGAGGAGGTT	AAAAAACCCCCAAAACAAAAA
<i>LHFPL3</i>	GAGGATGTAGATTTTGAAATTGGTG	AACTACCCCTACAAATCAACTCCC
<i>MPO</i>	GTTGGGGGTGGTTGTAGGAAT	CAACTAACCCCATACATAAACATAAA
<i>NAPEPLD</i>	TGTAATTTGGTAATTTGTAGGGAAGA	ATAACCTCTCTACTACCTCCTCCAC
<i>NFAT5</i>	AGATAGGGAGATAGGGAGATAGGGT	AAAAAATAATAACCCTACACTCAAAAC
<i>NUP133</i>	GGTTGGGAATATGATTTTAAGGAGT	TTCACAAAAAATTCAAAAACTACCA
<i>PBX1</i>	TTTTTGGAATTAAGAAATAGTGGAGAA	AAAATAATAAAAAACACAACAAACCCC
<i>PMPCB</i>	TGGTAAATATGTATTTTTTAGTAGTTGG	AAAATAAAACCCACCTCCCTACTC
<i>PRG2</i>	AGTGGGAGGTTTGTTTTAGTTTTT	AACCATTCTACTACCAAAAATACCCC
<i>PUS7</i>	GGGTTGGATTATAGAAGGTAGG	TAACCCTAACCCCAACCCCA
<i>RB1</i>	TTTTTTGAGGAGGATTTAGAGTAGG	CAAAATCCTATCACCATTCTACAAA
<i>RELN</i>	GTTTAGTTGTTGAAGGGGAAGGT	ACAACACAAATCACCATTTCCAAAC
<i>RINT1</i>	TTTTAATAAAGTGGAGGGGATTTTTAT	ATACCACCTCAATAAACCAACAATA
<i>RUNX1</i>	TTGGTTTTATGAATGAGAGTGTGTTG	ACCTACTTTCTTTTTCCAAATCTCC
<i>SLC12A8</i>	TAGTTAGGAGTTAGGTTTGGGGTTA	TAACAAATAAAAAACCAAAACACCC
<i>SLC25A13</i>	GTTAAGGTTGGGTTTAGTTAATGGG	AAAAATAACCCCCTCCCTCC
<i>SLC26A5</i>	AGAGTTTTTATGATGGTAGAGTATTTGT	CAATTCTACCTCTAACCCCTCTTCC
<i>TAL1</i>	GTTGGATTTTGTGTGGTTTTGTT	CCTAATAAATATACCCATTATCCTTTC
<i>TCF4</i>	GTTTATAAAGAGAAGGAGTT	ACCTAAAAATATCTCACTTC
<i>TLX3</i>	GGTTTAAGAAAGATGATATAGAGTTGT	ACTCAAATTCACACTATAAAATCCC
<i>ZNF25</i>	AAAATTGTTTATTAGTGTTTTTTGGAG	AAATCCTCTCCCCACCCTAAA

Each target region was PCR amplified in such a way that a T7 promoter was introduced at the reverse strand for subsequent *in vitro* transcription of the reverse strand. The resulting RNA was subjected to uracil-specific cleavage which yielded small fragments (termed CpG units) containing one to several CpG sites. The CpG methylation status translated into

difference in fragment weight due to C to T conversion after bisulfite treatment. The fragments were quantified by MALDI-TOF mass spectrometry. CpG units with low quality data (bad performance of methylation standards, low signal intensity or lack of data for more than 50% of the analyzed samples) were excluded.