

DNA methylation and leukemia susceptibility in China: Evidence from an updated meta-analysis

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Received November 14, 2014; Accepted May 20, 2016

DOI: 10.3892/mco.2016.959

Abstract. Mounting evidence supports a role for DNA methylation in the pathogenesis of leukemia; however, there no overview of these results in the Chinese population. The present study performed a comprehensive meta-analysis to establish candidate genes with an altered methylation status in Chinese leukemia patients. Eligible studies were identified through searching the National Center of Biotechnology Information PubMed and Wanfang databases. Studies were pooled and overall odds ratios with corresponding confidence intervals were calculated. A total of 4,325 leukemia patients and 2,010 controls from 94 studies on 53 genes were included in this meta-analysis, and 47 genes were found to be aberrantly methylated in leukemia patients. A further subgroup meta-analysis by leukemia subtype demonstrated that hypermethylation of 5 genes, namely cyclin-dependent kinase (CDKN)2A, DNA-binding protein inhibitor-4, CDKN2B, glioma pathogenesis-related protein 1 and p73, contributed to the risk of various subtypes of leukemia. In addition, a strong association between CDKN2A and leukemia was identified in Chinese (P<0.00001) but not in European patients. The aberrantly methylated genes identified in the present meta-analysis may help elucidate the mechanisms underlying the development of leukemia in Chinese patients.

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Key words: leukemia, DNA methylation, epigenetics, ethnic difference, Chinese population

Introduction

Leukemia is a complex hematological malignancy, characterised by clonal proliferation of malignant hematopoietic stem cells in the blood and bone marrow (1), with a total of 350,000 new cases and 25,700 deaths annually (2). Genetic as well as environmental factors have been suggested to be associated with leukemia, including trisomy 21, gender, cytotoxicity of anticancer drugs, exposure to benzene and ionising radiation (3-6). Leukemia is a heterogeneous disease that comprises acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML). ALL accounts for 81% of childhood leukemia cases, while CLL and AML frequently occur in adults (7).

Racial and ethnic disparities have been identified in the expression of leukemia-related genes, the clinical outcome and the mortality rate of leukemia (8-12). These disparities are likely due to a combination of genetic, environmental and socioeconomic factors (13), which may affect epigenetic changes. Epigenetics, such as DNA methylation, have been shown to play an important role in cancer susceptibility (14,15). Therefore, DNA methylation studies may help elucidate these racial and ethnic disparities in leukemia patients.

Aberrant DNA methylation of genes has been shown to be associated with a large number of human malignancies (16,17). Although a recent meta-analysis by our group identified significant associations between a number of aberrantly methylated genes and leukemia (18), the majority of the studies published in the Chinese language are overlooked. Thus, the aim of the present study was to focus on the association of aberrant DNA methylation and leukemia susceptibility in the Chinese population and to investigate ethnic differences in DNA methylation using subgroup meta-analyses.

Materials and methods

Selection of studies. A systematic literature search was performed through the National Center for Biotechnology Information (NCBI) PubMed and Wanfang literature databases, updated until July 10, 2014. The search was performed using the keywords 'leukemia' and 'methylation'. Potentially relevant articles were identified by their titles and abstracts, followed by selection of eligible studies based on full-text analysis. Case-control studies on gene methylation in Chinese leukemia patients containing sufficient information on methylation to calculate the odds ratios (ORs) and 95% confidence intervals (CIs) were considered to be eligible. A flow chart of the study selection process is shown in Fig. 1.

Data extraction. The following characteristics were extracted from each eligible study: First author's name, year of publication, disease category and methylation status of cases and controls. All the studies included were reviewed by three authors (D.J., Y.S. and C.X.). For genes with methylation data in other populations, the corresponding data were retrieved and subjected to meta-analyses for comparison with the Chinese population.

Statistical analysis. Review Manager 5.0 software (The Nordic Cochrane Centre, The Cochrane Collaboration, Copenhagen, Denmark) was used for the meta-analysis. The ORs and 95% CIs were calculated to evaluate the association between gene methylation and leukemia. Heterogeneity of the included studies was assessed using I² statistics (19). When there was significant heterogeneity (I²>50%), the random-effects model was used to calculate the overall OR and 95% CI; otherwise, the fixed-effects model was applied (20).

Results

Eligible studies. As shown in Fig. 1, 1,477 potentially relevant studies were identified for initial review from the NCBI PubMed and Wanfang literature databases. A total of 1,380 studies were excluded (1,036 irrelevant studies, 227 non-case-control studies and 117 studies with insufficient data). Finally, a total of 94 studies (61 studies on ALL, 76 on AML, 11 on CLL, 31 on CML and 3 other studies on leukemia), were included in the present meta-analysis (31,38,44-135). A total of 53 genes were identified among 4,325 leukemia patients and 2,010 control subjects, of which 19 were reported by only 1 study, 15 by 2 studies and 19 by \geq 3 studies (Tables I and II). For 47 of these 53 genes, aberrant methylation was proved to be significantly associated with leukemia.

Meta-analysis of the association between cyclin-dependent kinase (CDKN)2A methylation and leukemia. As shown in Fig. 2, 566 cases and 361 controls were included in the meta-analysis of CDKN2A methylation. The results indicated that hypermethylation of CDKN2A was a risk factor for leukemia (P<0.00001; OR=19.99; 95% CI: 11.37-35.17). Subgroup analysis by type of leukemia revealed that hypermethylation of CDKN2A was associated with an increased risk of AML (P<0.00001; OR=17.86; 95% CI: 7.79-40.93), ALL (P<0.00001; OR=24.01; 95% CI: 10.23-56.33) and CLL (P=0.04; OR=15.95; 95% CI: 1.16-218.94), but not of CML (P=0.08). However, there was no significant difference in the association results among different leukemia types (P=0.87).

Meta-analysis of the association between CDKN2B methylation and leukemia. The meta-analysis of the association between CDKN2B methylation and leukemia included 24 studies comprising 463 cases and 302 controls (Fig. 3).



Figure 1. Flow diagram of the study selection process.

The results revealed that hypermethylation of the *CDKN2B* gene was associated with the risk of leukemia (P<0.00001; OR=42.45; 95% CI=22.98-78.42). These 24 studies included 12 studies on ALL, 10 studies on AML and 2 studies on CML. A subtype meta-analysis revealed that *CDKN2B* promoter methylation was a risk factor for AML (P<0.00001; OR=54.11; 95% CI: 21.07-138.93), ALL (P<0.00001; OR=35.76; 95% CI: 14.92-85.69) and CML (P=0.004; OR=27.06; 95% CI: 2.88-254.55). There was no significant difference in the association results among different leukemia types (P=0.76).

Meta-analysis of the association between DNA-binding protein inhibitor-4 (*ID4*) *methylation and leukemia*. A total of 10 studies were included in the *ID4* methylation analysis (Fig. 4). The meta-analysis revealed that *ID4* methylation was a risk factor for leukemia (P<0.00001; OR=70.08; 95% CI: 24.12-203.64). Hypermethylation of the *ID4* gene was associated with an increased risk of AML (P<0.00001; OR=116.32; 95% CI: 25.40-532.59), ALL (P<0.00001; OR=104.68; 95% CI: 17.27-634.39), CML (P=0.002; OR=20.17; 95% CI: 3.05-133.21) and CLL (P=0.002; OR=693.00; 95% CI: 11.87-40460.96). There was no significant difference in the association results among different leukemia types (P=0.33).

Meta-analysis of the association between glioma pathogenesis-related protein 1 (GliPR1) methylation and leukemia. As shown in Fig. 5, the meta-analysis of the association between *GliPR1* methylation and leukemia included9 studies. The results revealed that *GliPR1* methylation was a risk factor for leukemia (P<0.00001; OR=6.45; 95% CI: 2.88-14.45). Hypermethylation of the *GliPR1* gene was associated with an increased risk of AML (P<0.00001; OR=30.33; 95% CI: 15.83-58.11), ALL (P<0.0001; OR=3.39; 95% CI: 1.88-6.13) and CML (P=0.006; OR=2.49; 95% CI: 1.30-4.77). Moreover, there was a significant difference in the association of *GliPR1* hypermethylation with the risk of leukemia among the different subtypes (P<0.00001).



Table I. Eligible case-control leukemia studies in the Chinese population.

Gene	No. of studies	Overall OR (95% CI)
CDKN2A	26	19.99 (11.37-35.17)
CDKN2B	24	42.45 (22.98-78.42)
ID4	10	70.08 (24.12-203.64)
GliPR1	9	6.45 (2.88-14.45)
p73	7	17.07 (6.20-47.02)
CT	6	46.85 (12.15-180.65)
DAPK	6	17.19 (5.43-54.41)
PRB	6	32.10 (9.19-112.16)
SFRP5	5	11.45 (3.19-41.12)
IGSF4	4	14.41 (3.21-64.72)
PRA	4	38.11 (8.14-178.29)
RASSEIA	4	22.62 (5.11-100.17)
SFRP2	4	30 28 (7 18-127 74)
LRP15	3	41 45 (6 63-259 10)
RIZ1	3	9 86 (1 84-52 78)
SFRP4	3	14 31 (2 77-73 90)
WT1	3	0.24 (0.10-0.54)
70-1	3	0.24 (0.10 - 0.54) 99 65 (18 14 - 547 54)
RARA	3	3 46 (0 65 18 30)
	2	08.28(5.22,1840,18)
	2	90.20 (J.22-1049.10) 10.18 (1.71.60.56)
	2	10.16(1.71-00.50) 41.25(5.41.216.24)
	2	41.33 (3.41-310.24)
	2	24.28 (3.13-187.20)
EDINKB	2	52.57 (5.92-270.80) 7.00 (2.71, 12.52)
FANCE	2	/.09 (5./1-15.55)
GKAF	2	04.30 (8.07-477.53)
HAGE	2	12.23 (1.00-90.39)
NPERS	2	83.30 (10.93-008.70) 74.00 (0.65.592.64)
mik-34B	2	74.99 (9.05-582.04)
RAGE-I	2	38.88 (3.23-287.87) 11.01 (1.45.07.86)
RUNX3	2	11.91 (1.45-97.86)
SFRPI	2	23.65 (3.05-183.57)
SHP1	2	11.05 (1.41-86.28)
WIF1	2	14.15 (1.78-112.81)
AKAP12	1	34.44 (1.85-640.43)
CDHI	1	33.00 (1./8-610.61)
CEBPZ	l	35.84 (2.12-604.80)
DRD4	1	27.26 (1.44-516.59)
E-cad	1	33.00 (1.78-610.61)
JUNB	1	55.00 (1.86-1622.60)
MT3	1	5.76 (1.17-28.24)
PLCD1	1	39.38 (2.21-702.41)
PRAME	1	42.49 (2.45-737.45)
PRDX2	1	36.82 (2.14-633.67)
RIL	1	197.19 (11.04-3523.69)
SOCS-1	1	34.26 (1.79-654.46)
WNT5A	1	121.51 (7.08-2085.83)
WWOX	1	41.70 (2.43-715.18)
DLC-1	1	17.52 (0.90-342.83)
p53	1	19.46 (0.92-411.20)
PDLIM4	1	14.23 (0.81-249.59)

Table I. Continued.

Gene	No. of studies	Overall OR (95% CI)
PTEN	1	16.03 (0.83-308.79)
SALL4	1	10.67 (0.59-192.94)

OR, odds ratio; CI, confidence interval.

Meta-analysis of the association between p73 methylation and leukemia. The meta-analysis of p73 methylation included 7 case-control studies (Fig. 6). The results revealed that hypermethylation of p73 was associated with an increased risk of leukemia (P<0.00001; OR=17.07; 95% CI: 6.20-47.02). In addition, the results showed that p73 methylation was a risk factor for AML (P=0.002; OR=20.83; 95% CI: 3.01-143.95) and ALL (P<0.00001; OR=15.92; 95% CI: 4.87-52.07), while there was no significant difference between the two subtypes.

Subgroup meta-analysis of gene methylation and leukemia by ethnicity. Based on our previous study (18), a further subgroup meta-analysis by ethnicity was performed for *CDKN2A* and *CDKN2B* methylation. Hypermethylation of *CDKN2A* and *CDKN2B* was associated with an increased risk of leukemia in Chinese populations (P<0.00001), while only *CDKN2B* was associated with leukemia in Europeans (P=0.007) (Figs. 7 and 8). Of note, there was a significant difference between European and Chinese populations regarding the association of *CDKN2A* and *CDKN2B* methylation with leukemia (P<0.00001 and P=0.02, respectively).

Discussion

In the present study, eligible studies were retrieved from the NCBI PubMed and Wanfang literature databases and a systematic meta-analysis was performed to investigate the association between the methylation status of 53 genes and leukemia, with the aim of providing evidence regarding the role of gene methylation in the pathogenesis of leukemia, particularly in different leukemia subgroups and ethnic groups.

Aberrant gene promoter methylation, occurring in almost every tumor type, is one of several mechanisms of gene inactivation (21). Promoter hypermethylation of tumor suppressor genes often contributes to loss of function and cancer development (22,23). One potential mechanism for hypermethylation-induced silencing is changing the structure of specific binding sites for certain transcriptional regulators (24). Epigenetic silencing of genes by promoter hypermethylation is associated with the loss of tumor suppression, increasing tumor severity and reducing patient survival (25). The present meta-analysis revealed significant changes in the methylation status of the *CDKN2A*, *CDKN2B*, *ID4*, *GliPR1*, *p73* and Wilms' tumor 1 (*WT1*) genes in the major types of leukemia (21,23,26-28).

Numerous studies revealed that *CDKN2A* and *CDKN2B* methylation is frequent during malignant transformation (29-31). As tumor suppressors, *CDKN2A* and *CDKN2B* generate 3 transcript variants (p16^{INK4A}, p14^{ARF} and p15^{INK2B})

				Case	es (n)	Contr		
Authors	Year	Gene	Disease	Meth	Total	Meth	Total	Refs.
Hsiao <i>et al</i>	2008	CDKN2A	ALL	11	13	0	8	(31)
Hsiao <i>et al</i>	2008	CDKN2A	CLL	1	1	0	8	(31)
Hsiao <i>et al</i>	2008	CDKN2A	AML	5	6	0	8	(31)
Hsiao <i>et al</i>	2008	CDKN2A	CML	1	3	0	8	(31)
Zheng et al	2004	CDKN2A	ALL	12	20	0	20	(32)
Xiao <i>et al</i>	2010	CDKN2A	AML	7	21	0	16	(33)
Xiao <i>et al</i>	2010	CDKN2A	ALL	7	17	0	16	(33)
Xiao <i>et al</i>	2010	CDKN2A	CML	1	7	0	16	(33)
Xiao <i>et al</i>	2010	CDKN2A	CLL	1	6	0	16	(33)
Yang <i>et al</i>	2003	CDKN2A	ALL	5	28	0	20	(34)
Yang <i>et al</i>	2003	CDKN2A	AML	9	43	0	20	(34)
Song <i>et al</i>	2004	CDKN2A	ALL	5	28	0	20	(35)
Tan <i>et al</i>	2001	CDKN2A	AML	14	20	0	20	(36)
Zhu <i>et al</i>	2005	CDKN2A	ALL	8	19	0	10	(37)
Zhang <i>et al</i>	2000	CDKN2A	ALL	20	40	0	15	(38)
Fan <i>et al</i>	2007	CDKN2A	AML	24	58	0	16	(39)
Fan <i>et al</i>	2007	CDKN2A	ALL	8	24	0	16	(39)
Jiang <i>et al</i>	2002	CDKN2A	ALL	19	31	0	20	(40)
Jiang <i>et al</i>	2002	CDKN2A	AML	14	18	0	20	(40)
Meng <i>et al</i>	2005	CDKN2A	AML	3	26	0	10	(41)
Meng <i>et al</i>	2005	CDKN2A	ALL	2	14	0	10	(41)
Wang <i>et al</i>	2002	CDKN2A	ALL	11	15	0	12	(42)
Yin <i>et al</i>	2002	CDKN2A	ALL	6	15	0	12	(43)
Chen <i>et al</i>	2003	CDKN2A (HapII)	AML	11	31	0	8	(44)
Chen <i>et al</i>	2003	CDKN2A (NruI)	AML	22	31	2	8	(44)
Chen <i>et al</i>	2003	CDKN2A (SacII)	AML	19	31	1	8	(44)
Lin <i>et al</i>	2012	CDKN2B	ALL	17	25	0	10	(45)
Zheng <i>et al</i>	2004	CDKN2B	ALL	18	26	0	20	(32)
Chen <i>et al</i>	2003	CDKN2B	AML	16	31	0	8	(44)
Tan <i>et al</i>	2001	CDKN2B	AML	16	20	0	20	(36)
Zhu <i>et al</i>	2001	CDKN2B	ALL	12	20	0	10	(46)
Zhu <i>et al</i>	2005	CDKN2B	ALL	7	19	0	10	(37)
Shen <i>et al</i>	2002	CDKN2B	ALL	6	10	0	10	(47)
Shen <i>et al</i>	2002	CDKN2B	AML	10	25	0	10	(47)
Fan <i>et al</i>	2002	CDKN2B	CML	5	25 7	0	20	(48)
Tong <i>et al</i>	2004	CDKN2B	AMI	5	10	0	10	(10)
Tong <i>et al</i>	2004	CDKN2B	ALI	4	10	0	10	(49)
Tong <i>et al</i>	2004	CDKN2B	CMI	5	10	0	10	(49)
Guo <i>et al</i>	2004	CDKN2B	AMI	26	31	0	30	(50)
Oizo et al	2000	CDKN2B CDKN2B		20 34	42	0	30 14	(50)
Qiao et al	2005	CDKN2B CDKN2B		0	42	0	14	(51)
Chen <i>et al</i>	2005	CDKN2B CDKN2B		5	14	0	14	(51)
Vin <i>et al</i>	2000	CDKN2B CDKN2B		5	10	0	10	(52)
Vin <i>et al</i>	2003	CDKN2B CDKN2B		17	22	0	12	(53)
Meng et al	2005	CDKN2B CDKN2B		24	26	0	10	(33)
Meng <i>et al</i>	2005	CDKN2B CDKN2B		10	20 14	0	10	(41)
Wu et al	2003	CDKN2P		10	14	0	10	(+1) (54)
Wu et al	2013	CDKN2D CDKN2P		1 4 6	14	0	14	(54)
Wang et al	2013	CDKN2D CDKN2P		7	10	0	14	(54)
Wang et al	2002	CDKN2D CDKN2P		22	22	0	7	(55)
wang ei ül	2002	CDMN2D	ANL		55	U	1	(33)

Table II. List of methylated	genes and	associated	case-control	studies.
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Table II. Continued.

				Case	es (n)	Contr	ols (n)	
Authors	Year	Gene	Disease	Meth	Total	Meth	Total	Refs.
Zhao et al	2008	ID4	AML	15	32	0	18	(56)
Wang <i>et al</i>	2010	ID4	CML	6	48	0	10	(57)
Liu et al	2011	ID4	AML	39	46	0	10	(58)
Jie et al	2012	ID4	AML	21	23	1	20	(59)
Jie et al	2012	ID4	ALL	9	13	1	20	(59)
Jie et al	2012	ID4	CML	9	11	1	20	(59)
Zhao <i>et al</i>	2005	ID4	AML	21	25	0	49	(60)
Zhao <i>et al</i>	2005	ID4	CML	2	4	0	49	(60)
Zhao <i>et al</i>	2005	ID4	ALL	12	14	0	49	(60)
Zhao <i>et al</i>	2005	ID4	CLL	3	3	0	49	(60)
Xiao <i>et al</i>	2011	GLIPR1	AML	58	70	14	93	(61)
Xiao <i>et al</i>	2011	GLIPR1	CML	11	40	14	93	(61)
Xiao <i>et al</i>	2011	GLIPR1	ALL	22	57	14	93	(61)
Liang <i>et al</i>	2009	GLIPR1	AML	44	54	5	35	(62)
Liang <i>et al</i>	2009	GLIPR1	CML	11	40	5	35	(62)
Liang <i>et al</i>	2009	GLIPR1	ALL	18	48	5	35	(62)
Jie <i>et al</i>	2012	GLIPR1	AML	22	23	4	20	(59)
Jie et al	2012	GLIPR1	ALL	5	13	4	20	(59)
Jie et al	2012	GLIPR1	CML	6	11	4	20	(59)
Zhang <i>et al</i>	2010	p73	AML	1	30	1	123	(63)
Zhang <i>et al</i>	2010	p73	ALL	10	112	1	123	(63)
Zhang <i>et al</i>	2012	p73	AML	21	58	0	31	(64)
Wu et al	2008	p73	ALL	10	30	0	16	(65)
Liu et al	2005	p73	ALL	10	26	0	18	(66)
Xu et al	2005	p73	ALL	12	42	0	10	(67)
Yu et al	2014	p73	ALL	10	32	0	30	(68)
Xie et al	2003	CT	AML	25	31	0	14	(69)
Xie et al	2003	CT	CML	13	45	0	14	(69)
Tang <i>et al</i>	2001	CT	CLL	1	3	0	30	(70)
Tang <i>et al</i>	2001	CT	CML	8	10	0	30	(70)
Tang <i>et al</i>	2001	CT	ALL	12	14	0	30	(70)
Wang <i>et al</i>	1998	CT	CML	13	31	0	10	(71)
Qian et al	2010	DAPK	AML	82	112	0	15	(72)
Niu et al	2014	DAPK	AML	33	102	0	7	(73)
Niu et al	2014	DAPK	ALL	8	17	0	7	(73)
Zhao <i>et al</i>	2009	DAPK	AML	3	60	0	17	(74)
Zhao <i>et al</i>	2009	DAPK	ALL	16	55	0	17	(74)
Qian J	2008	DAPK	CML	25	49	0	13	(75)
Lin W	2010	PRB	CLL	18	27	0	15	(76)
Wu B	2008	PRB	CLL	5	9	0	5	(77)
Zhang <i>et al</i>	2003	PRB	ALL	6	11	0	10	(78)
Zhang <i>et al</i>	2003	PRB	CLL	6	8	0	10	(78)
Zhang <i>et al</i>	2003	PRB	AML	9	15	0	10	(78)
Zhang <i>et al</i>	2003	PRB	CML	6	10	0	10	(78)
Shi et al	2011	SFRP5	AML	10	99	1	70	(79)
Wang <i>et al</i>	2012	SFRP5	CML	3	3	0	6	(80)
Wang et al	2012	SFRP5	AML	4	7	0	6	(80)
Xu et al	2010	SFRP5	AML	6	59	0	20	(81)
Xu et al	2010	SFRP5	ALL	9	28	0	20	(81)
Li et al	2004	IGSF4	AML	16	29	0	8	(82)

Table II. Continued.

				Case	es (n)	Contr		
Authors	Year	Gene	Disease	Meth	Total	Meth	Total	Refs.
Li et al	2004	IGSF4	ALL	12	21	0	8	(82)
Li et al	2004	IGSF4	CML	6	18	0	8	(82)
Li et al	2004	IGSF4	CLL	2	7	0	8	(82)
Zhang <i>et al</i>	2003	PRA	ALL	7	11	0	10	(78)
Zhang <i>et al</i>	2003	PRA	CLL	5	8	0	10	(78)
Zhang <i>et al</i>	2003	PRA	AML	10	15	0	10	(78)
Zhang <i>et al</i>	2003	PRA	CML	7	10	0	10	(78)
Chen <i>et al</i>	2012	RASSF1A	AML	2	24	0	60	(83)
Chen et al	2012	RASSF1A	CML	1	23	0	60	(83)
Chen et al	2012	RASSF1A	ALL	5	19	0	60	(83)
Chen et al	2012	RASSF1A	CLL	4	20	0	60	(83)
Song <i>et al</i>	2011	SFRP2	CML	25	38	0	13	(84)
Shi <i>et al</i>	2011	SFRP2	AML	27	99	0	70	(79)
Xu et al	2010	SFRP2	AML	14	59	0	20	(81)
Xu et al	2010	SFRP2	ALL	8	28	0	20	(81)
Dou <i>et al</i>	2004	LRP15	AML	37	53	0	9	(85)
Dou <i>et al</i>	2004	LRP15	ALL	15	20	0	9	(85)
Dou <i>et al</i>	2004	LRP15	CLL	1	2	0	9	(85)
Yao <i>et al</i>	2010	RIZ1	AML	11	37	0	15	(86)
Cai <i>et al</i>	2012	RIZ1	ALL	15	64	0	9	(87)
Cai <i>et al</i>	2012	RIZ1	AML	12	32	0	9	(87)
Shi et al	2011	SFRP4	AML	17	99	0	70	(88)
Xu et al	2010	SFRP4	AML	4	59	0	20	(81)
Xu et al	2010	SFRP4	ALL	7	28	0	20	(81)
Jie <i>et al</i>	2012	WT1	AML	8	23	15	20	(59)
Jie <i>et al</i>	2012	WT1	ALL	4	13	15	20	(59)
Jie <i>et al</i>	2012	WT1	CML	7	11	15	20	(59)
Dou <i>et al</i>	2009	ZO-1	Leukemia	7	10	0	10	(89)
Wang <i>et al</i>	2008	ZO-1	AML	32	52	0	40	(90)
Wang <i>et al</i>	2008	ZO-1	ALL	17	29	0	40	(90)
Chim <i>et al</i>	2005	RARA	APL	25	63	0	8	(91)
Chim et al	2005	RARA	AML	1	50	0	8	(91)
Chim et al	2005	RARA	ALL	1	25	0	8	(91)
Wang <i>et al</i>	2007	AR	ALL	4	4	0	3	(92)
Wang <i>et al</i>	2007	AR	AML	11	11	0	3	(92)
Wang <i>et al</i>	2009	CDH13	CML	4	8	0	5	(93)
Liu <i>et al</i>	2013	CDH13	AML	23	44	1	10	(94)
Wang <i>et al</i>	2009	DDIT3	AML	62	133	0	16	(95)
Wang <i>et al</i>	2009	DDIT3	CML	39	59	0	16	(95)
Zhu <i>et al</i>	2012	DKK-1	ALL	14	34	0	20	(96)
Zhu <i>et al</i>	2012	DKK-1	AML	10	31	0	20	(96)
Yuan <i>et al</i>	2010	EDNRB	AML	15	22	0	8	(97)
Yuan <i>et al</i>	2010	EDNRB	ALL	11	17	0	8	(97)
Yu et al	2008	FANCF	AML	41	58	7	20	(98)
Deng <i>et al</i>	2009	FANCF	AML	85	111	11	42	(99)
Qian <i>et al</i>	2010	GRAF	AML	87	132	0	20	(100)
Qian <i>et al</i>	2010	GRAF	CML	34	61	0	20	(100)
Chen et al	2012	HAGE	AML	32	214	0	24	(101)
Chen et al	2012	HAGE	CML	22	87	0	24	(101)
Li et al	2011	hPER3	CML	12	29	0	40	(102)



Table II. Continued.

				Case	es (n)	Contr		
Authors	Year	Gene	Disease	Meth	Total	Meth	Total	Refs.
Wang <i>et al</i>	2011	hPER3	AML	116	206	0	40	(103)
Wang <i>et al</i>	2013	miR-34B	ALL	24	31	0	23	(104)
Wang et al	2013	miR-34B	AML	8	19	0	23	(104)
Chai <i>et al</i>	2013	RAGE-1	AML	52	121	0	25	(105)
Chai <i>et al</i>	2013	RAGE-1	CML	33	76	0	25	(105)
Lin et al	2008	RUNX3	AML	7	23	0	10	(106)
Lin et al	2008	RUNX3	ALL	7	17	0	10	(106)
Xu et al	2010	SFRP1	AML	20	59	0	20	(81)
Xu et al	2010	SFRP1	ALL	11	28	0	20	(81)
Chim et al	2004	SHP1	AML	26	50	0	8	(107)
Chim et al	2004	SHP1	ALL	6	25	0	8	(107)
Wang <i>et al</i>	2011	WIF1	AML	11	34	0	15	(108)
Wang <i>et al</i>	2011	WIF1	ALL	6	21	0	15	(108)
Liu et al	2008	AKAP12	ALL	20	32	0	10	(109)
Gao et al	2006	CDH1	AML	38	55	0	7	(110)
Yao <i>et al</i>	2011	CEBPZ	AML	62	133	0	20	(111)
Guan <i>et al</i>	2008	DLC-1	ALL	21	34	0	5	(112)
Yu et al	2000	DRD4	AML	16	27	0	9	(113)
Gao et al	2006	E-cad	AML	38	55	0	7	(110)
Wang et al	2009	JUNB	CML	7	8	0	5	(93)
Tao <i>et al</i>	2014	MT3	AML	16	41	2	20	(114)
Zheng et al	2007	p53	ALL	5	11	0	11	(115)
Li et al	2013	PDLIM4	CML	13	59	0	24	(116)
Song et al	2012	PLCD1	CML	23	41	0	15	(117)
Yao <i>et al</i>	2013	PRAME	CML	28	55	0	20	(118)
Yan <i>et al</i>	2012	PRDX2	AML	17	55	0	40	(119)
Yang <i>et al</i>	2007	PTEN	ALL	5	22	0	25	(120)
Du et al	2013	RIL	AML	50	60	0	20	(121)
Jiao <i>et al</i>	2013	SALL4	AML	9	45	0	20	(122)
Zhuang <i>et al</i>	2011	SOCS-1	AML	15	24	0	10	(123)
Deng et al	2011	WNT5A	Leukemia	47	68	0	27	(124)
Zhang <i>et al</i>	2012	WWOX	AML	23	58	0	31	(64)

ALL, acute lymphocytic leukemia; CLL, chronic lymphocytic leukemia; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; Meth, methylated.

according to differences in the first exons and control the progression of cells from the Gl to the S phase (29,125). The present meta-analysis demonstrated that hypermethylation of *CDKN2A* and *CDKN2B* are risk factors for leukemia. According to the subgroup meta-analysis, hypermethylation of *CDKN2A* was significantly associated with AML, ALL and CLL, but not with CML, while *CDKN2B* hypermethylation was significantly associated with AML, ALL and CML. The lack of association of *CDKN2A* with CML may be attributed to the limited sample included in the the meta-analyses (CML power, 6.4%; and CLL power, 6.3%).

The ID4 protein is a member of the dominant-negative basic helix-loop-helix transcription factor family that lacks DNA-binding activity (126) and has a tumor suppressor function. The promoter of *ID4* was reported to be consistently methylated to various degrees in CLL and a univariate analysis demonstrated that increased promoter methylation of *ID4* was correlated with shortened patient survival (127). Previous studies also reported that *ID4* gene promoter hypermethylation was highly correlated with acute leukemia and may reflect the malignant degree of AML (128,129). The results of the present meta-analysis demonstrated that methylation of the *ID4* gene was associated with an increased risk of leukemia, particularly CML.

The GliPR1 protein, encoded by the *GliPR1* gene, has been identified as an epigenetically regulated tumor suppressor in prostate cancer and AML. *GliPR1* may serve as a marker for monitoring disease activity in AML patients during

	Case	•	Contro	ol		Odds Ratio		Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% C	Year	M-H, Fixed, 95% Cl
2.1.1 AML								
Tan Guangxiao 2001	14	20	0	20	1.7%	91.46 [4.77, 1754.50]	2001	$ \longrightarrow$
Jiang Junhuang 2002	14	18	0	20	1.3%	132.11 [6.59, 2648.61]	2002	
Chen Hui 2003 (Sac II)	19	31	1	8	6.9%	11.08 [1.21, 101.68]	2003	
Chen Hui 2003 (Nrul)	22	31	2	8	10.3%	7.33 [1.24, 43.41]	2003	
Yang Junzheng 2003	9	43	0	20	5.9%	11.29 [0.62, 204.34]	2003	
Chen Hui 2003 (Hap II)	11	31	0	8	5.6%	9.54 [0.50, 180.78]	2003	
Meng Yuesheng 2005	3	26	0	10	6.9%	3.13 [0.15, 66.12]	2005	
Fan Liping 2007	24	58	0	16	5.1%	23.43 [1.34, 409.51]	2007	→
Pei-Ching Hsiao 2008	5	6	0	8	1.0%	62.33 [2.13, 1822.63]	2008	
Xiao Yun 2010	7	21	0	16	4.1%	17.07 [0.89, 325.59]	2010	
Subtotal (95% CI)		285		134	48.7%	17.86 [7.79, 40.93]		•
Total events	128		3					
Heterogeneity: Chi ² = 6.11	, df = 9 (F	P = 0.73	3); l ² = 0%					
Test for overall effect: Z =	6.81 (P <	0.0000	01)					
2.1.2 ALL								
Zhang Junli 2000	20	40	0	15	4.0%	31.00 [1.74, 553.31]	2000	$ \longrightarrow$
Jiang Junhuang 2002	19	31	0	20	2.6%	63.96 [3.54, 1155.34]	2002	$ \longrightarrow$
Yin Yumin 2002	6	15	0	12	3.6%	17.11 [0.85, 342.74]	2002	· · · · · · · · · · · · · · · · · · ·
Wang Boxun 2002	11	15	0	12	1.7%	63.89 [3.09, 1321.68]	2002	$ \longrightarrow$
Yang Junzheng 2003	5	28	0	20	5.2%	9.60 [0.50, 184.26]	2003	
Zheng Ruiji 2004	12	20	0	20	2.3%	60.29 [3.20, 1137.79]	2004	$ \longrightarrow$
Song Guoying 2004	5	28	0	20	5.2%	9.60 [0.50, 184.26]	2004	
Zhu Chuansheng 2005	8	19	0	10	4.1%	15.52 [0.79, 303.25]	2005	<u>+</u> →
Meng Yuesheng 2005	2	14	0	10	5.4%	4.20 [0.18, 97.55]	2005	
Fan Liping 2007	8	24	0	16	4.4%	17.00 [0.91, 319.22]	2007	
Pei-Ching Hsiao 2008	11	13	0	8	1.2%	78.20 [3.31, 1849.02]	2008	$ \longrightarrow$
Xiao Yun 2010	7	17	0	16	3.3%	23.57 [1.21, 457.36]	2010	
Subtotal (95% CI)		264		179	43.2%	24.01 [10.23, 56.33]		•
Total events	114		0					
Heterogeneity: Chi ² = 3.89	, df = 11	(P = 0.9)	97); l ² = 09	6				
Test for overall effect: Z =	7.30 (P <	0.0000	01)					
2.1.3 CML								
Pei-Ching Hsiao 2008	1	3	0	8	2.1%	10.20 [0.31, 336.93]	2008	
Xiao Yun 2010	1	7	0	16	2.9%	7.62 [0.27, 212.08]	2010	
Subtotal (95% CI)		10		24	5.0%	8.71 [0.78, 97.22]		
Total events	2		0					
Heterogeneity: Chi ² = 0.01	, df = 1 (F	^o = 0.9'	1); l ² = 0%					
Test for overall effect: Z =	1.76 (P =	0.08)						
2.1.4 CLL								
Pei-Ching Hsiao 2008	1	1	0	8	0.5%	51.00 [0.70, 3710.31]	2008	+
Xiao Yun 2010	1	6	0	16	2.6%	9.00 [0.32, 254.72]	2010	
Subtotal (95% CI)		7		24	3.1%	15.95 [1.16, 218.94]		
Total events	2		0					
Heterogeneity: Chi ² = 0.39	, df = 1 (F	P = 0.53	3); ² = 0%					
Test for overall effect: Z =	2.07 (P =	0.04)						
Total (95% CI)		566		361	100.0%	19.99 [11.37, 35.17]		•
Total events	246		3					
Heterogeneity: Chi ² = 11.1	9, df = 25	6 (P = 0	.99); ² = ()%				
Test for overall effect: Z =	10.40 (P	< 0.000	001)					Decreased risk Increased risk
Test for subaroup difference	ces: Chi ²	= 0.72.	df = 3 (P	= 0.87). ² = 0%			Decreased lisk Increased lisk

Figure 2. Meta-analyses of aberrantly methylated cyclin-dependent kinase 2A gene in leukemia. ALL, acute lymphocytic leukemia; CLL, chronic lymphocytic leukemia; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; M-H, Mantel-Haenszel model; CI, confidence interval; df, degree of freedom.

therapy (61,130). Moreover, *GliPR1* expression was found to be significantly increased in bone marrow samples of AML patients, while being markedly reduced in ALL, unchanged in myelodysplastic syndrome and marginally decreased in CLL and CML (131). The present meta-analysis identified hypermethylation of the *GliPR1* promoter as a risk factor for leukemia in the Chinese population.

p73, a homologue of the p53 tumor suppressor family, is involved in neurogenesis, sensory pathways, immunity, inflammation and tumorigenesis (132). Furthermore, p73



	Case		Contro	bl		Odds Ratio		Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% C	l Year	M-H, Fixed, 95% Cl
1.1.1 AML								
Guo Xiuzhi 2000	26	31	0	30	1.6%	293.91 [15.51, 5567.75]	2000	
Tan Guangxiao 2001	16	20	0	20	2.0%	150.33 [7.54, 2997.83]	2001	
Shen Zhijian 2002	10	25	0	10	7.8%	14.23 [0.75, 269.96]	2002	
Wang Haiyan 2002	22	33	0	7	5.1%	29.35 [1.54, 560.47]	2002	$ \longrightarrow$
Yin Yumin 2003	17	22	0	12	2.9%	79.55 [4.02, 1573.39]	2003	$ \longrightarrow$
Chen Hui 2003	16	31	0	8	7.1%	18.10 [0.96, 340.68]	2003	
Tong Hongyan 2004	5	10	0	10	4.7%	21.00 [0.97, 453.91]	2004	
Qiao Shukai 2005	34	42	0	14	2.7%	117.71 [6.36, 2176.72]	2005	$ \longrightarrow$
Meng Yuesheng 2005	24	26	0	10	1.2%	205.80 [9.08, 4666.41]	2005	
Wu Dansen 2013	6	14	0	14	5.3%	22.18 [1.11, 444.74]	2013	
Subtotal (95% CI)		254		135	40.5%	54.11 [21.07, 138.93]		
Total events	176		0					
Heterogeneity: Chi ² = 4.96	6, df = 9 (F	P = 0.8	4); I ² = 0%	,				
Test for overall effect: Z =	8.30 (P <	0.000	D1)					
1.1.2 ALL								
Chen Fei 2000	5	10	0	10	4.7%	21.00 [0.97, 453.91]	2000	
Zhu Chuansheng 2001	12	21	0	10	5.4%	27.63 [1.43, 533.13]	2001	
Wang Haiyan 2002	7	10	0	7	3.4%	32.14 [1.40, 736.17]	2002	 →
Shen Zhijian 2002	6	10	0	10	3.8%	30.33 [1.39, 660.76]	2002	$ \longrightarrow$
Yin Yumin 2003	6	15	0	12	6.1%	17.11 [0.85, 342.74]	2003	<u> </u>
Tong Hongyan 2004	4	10	0	10	5.5%	14.54 [0.67, 316.69]	2004	
Zheng Ruiji 2004	18	26	0	20	3.3%	89.24 [4.81, 1655.53]	2004	$ \longrightarrow $
Zhu Chuansheng 2005	7	19	0	10	7.5%	12.60 [0.64, 247.56]	2005	
Meng Yuesheng 2005	10	14	0	10	3.2%	49.00 [2.33, 1028.86]	2005	$ \longrightarrow$
Qiao Shukai 2005	9	14	0	14	3.4%	50.09 [2.47, 1014.62]	2005	$ \longrightarrow$
Lin Fuan 2012	17	25	0	10	4.3%	43.24 [2.26, 828.53]	2012	$ \longrightarrow$
Wu Dansen 2013	14	14	0	14	0.3%	841.00 [15.61, 45321.27]	2013	
Subtotal (95% CI)		188		137	51.1%	35.76 [14.92, 85.69]		•
Total events	115		0					
Heterogeneity: Chi ² = 4.08	3, df = 11 (P = 0.	97); l² = 0	%				
Test for overall effect: Z =	8.02 (P <	0.000	D1)					
1.1.3 CML								
Fan Hongtao 2001	5	7	0	20	1.6%	90.20 [3.76, 2166.61]	2001	$ \longrightarrow$
Tong Hongyan 2004	5	14	0	10	6.8%	12.16 [0.59, 250.42]	2004	
Subtotal (95% CI)		21		30	8.4%	27.06 [2.88, 254.55]		
Total events	10		0					
Heterogeneity: Chi ² = 0.82	2, df = 1 (F	P = 0.3	7); I ² = 0%	,				
Test for overall effect: Z =	2.88 (P =	0.004)					
Total (95% CI)		463		302	100.0%	42.45 [22.98. 78.42]		•
Total events	301	100	0			and functor (other]		· ·
Heterogeneity: Chi ² = 10.2	38 df = 23	(P = 0)	1 99)· 12 - 1	0%				+ + + + + + + + + + + + + + + + + + + +
Test for overall effect: 7 -	11 07 (D		n01)	0 /0				0.005 0.1 1 10 200
Test for subgroup differen	cos: Chi2	= 0.56	df = 2/P	= 0.76	$1^2 = 0^{0/2}$			Decreased risk Increased risk

Figure 3. Meta-analyses of aberrantly methylated cyclin-dependent kinase 2B gene in leukemia. ALL, acute lymphocytic leukemia; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; M-H, Mantel-Haenszel model; CI, confidence interval; df, degree of freedom.

hypermethylation resulting in its deactivation is frequently observed in malignant lymphoproliferative disorders, particularly ALL (21). In line with these results, the present meta-analysis also identified p73 hypermethylation as a risk factor for leukemia in the Chinese population.

The *WT1* gene encodes a zinc finger transcription factor that is an RNA-binding protein with important roles in the development of several organs and tissues. *WT1* has been reported to have tumor suppressor as well as oncogenic activity; however. the reasons and mechanisms underlying these opposing functions remain to be fully elucidated (133). The present study demonstrated that *WT1* hypermethylation played a protective role against the progression of leukemia. Previous studies have reported that the risk of hematological malignancies varies significantly among different ethnic groups (9,13,134,135). The present meta-analysis indicated that there was no association between CDKN2A methylation and the risk of leukemia (P=0.16) in Europeans, while a significant association was observed in Chinese populations (P<0.00001). A significant difference in the association of CDKN2A methylation with leukemia was observed between European and Chinese populations (P<0.00001). This result may provide molecular evidence to guide future individualization of chemotherapy for leukemia, although further research is required to elucidate the precise nature of the ethnic differences in leukemia.

	Case	•	Contr	ol		Odds Ratio			Odds	Ratio	
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% C	Year		M-H, Fix	ed, 95% Cl	
3.1.1 AML											
Zhao Yu 2005	21	25	0	49	3.3%	473.00 [24.38, 9176.21]	2005			-	
Zhao Yu 2008	15	32	0	18	18.8%	32.77 [1.82, 590.26]	2008				
Liu Fei 2011	39	46	0	10	7.2%	110.60 [5.83, 2097.30]	2011				
Jie Xiaomei 2012	21	23	1	20	5.2%	199.50 [16.72, 2380.79]	2012				\rightarrow
Subtotal (95% CI)		126		97	34.5%	116.32 [25.40, 532.59]				◀	
Total events	96		1								
Heterogeneity: Chi ² = 1	.78, df = 3	B (P = 0	.62); l ² =	0%							
Test for overall effect: 2	Z = 6.13 (F	o < 0.00	0001)								
3.1.2 ALL											
Zhao Yu 2005	12	14	0	49	2.1%	495.00 [22.32, 10978.11]	2005			-	
Jie Xiaomei 2012	9	13	1	20	13.5%	42.75 [4.16, 439.56]	2012				
Subtotal (95% CI)		27		69	15.7%	104.68 [17.27, 634.39]					
Total events	21		1								
Heterogeneity: Chi ² = 1	.53, df = 1	(P=0	.22); l ² = 3	35%							
Test for overall effect: 2	z = 5.06 (F	< 0.00	0001)								
3.1.3 CML											
Zhao Yu 2005	2	4	0	49	2.5%	99.00 [3.68, 2661.32]	2005				
Wang XinRong 2010	6	48	0	10	39.5%	3.21 [0.17, 61.65]	2010				
Jie Xiaomei 2012	9	11	1	20	7.2%	85.50 [6.82, 1071.27]	2012				• • •
Subtotal (95% CI)		63		79	49.3%	20.17 [3.05, 133.21]					
Total events	17		1								
Heterogeneity: Chi ² = 3	8.64, df = 2	2 (P = 0).16); l ² = 4	45%							
Test for overall effect: 2	Z = 3.12 (F	P = 0.00	02)								
3.1.4 CLL											
Zhao Yu 2005	3	3	0	49	0.5%	693.00 [11.87, 40460.96]	2005				
Subtotal (95% CI)		3		49	0.5%	693.00 [11.87, 40460.96]					
Total events	3		0								
Heterogeneity: Not app	licable										
Test for overall effect: 2	Z = 3.15 (F	P = 0.00	02)								
Total (95% CI)		219		294	100.0%	70.08 [24.12, 203.64]					
Total events	137		3								
Heterogeneity: Chi ² = 9	0.80, df = 9) (P = 0).37); l ² = 3	8%				0.001	0.1	1 10	1000
Test for overall effect: 2	2 = 7.81 (F	< 0.00	JUU1)	(D - C	00) 12 -	12.00/		Decrea	ased risk	Increased	risk
lest for subaroup differ	rences: Cr	$n^{-} = 3.4$	45. af = 3	(P = 0.	33), ² = '	13.0%					

Figure 4. Meta-analyses of aberrantly methylated DNA-binding protein inhibitor-4 gene in leukemia. ALL, acute lymphocytic leukemia; CLL, chronic lymphocytic leukemia; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; M-H, Mantel-Haenszel model; CI, confidence interval; df, degree of freedom.

	Cas	е	Contr	ol		Odds Ratio		Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, 95% CI	Year	M-H, 95% CI
5.1.1 AML								
Liang Ting 2009	44	54	5	35	4.2%	26.40 [8.20, 85.02]	2009	
Yan-Hua Xiao 2011	58	70	14	93	7.7%	27.27 [11.75, 63.32]	2011	
Jie Xiaomei 2012	22	23	4	20	0.7%	88.00 [8.97, 863.77]	2012	
Subtotal (95% CI)		147		148	12.6%	30.33 [15.83, 58.11]		•
Total events	124		23					
Heterogeneity: Chi ² =	0.95, df=	2 (P =	0.62); I ² =	= 0%				
Test for overall effect:	Z = 10.29	(P < 0.	.00001)					
5.1.2 CML								
Liang Ting 2009	11	40	5	35	14.5%	2.28 [0.70, 7.36]	2009	+
Yan-Hua Xiao 2011	11	40	14	93	22.8%	2.14 [0.87, 5.25]	2011	⊢ ∎
Jie Xiaomei 2012	6	11	4	20	4.8%	4.80 [0.95, 24.14]	2012	
Subtotal (95% CI)		91		148	42.1%	2.49 [1.30, 4.77]		
Total events	28		23					
Heterogeneity: Chi ² =	0.77, df=	2 (P =	0.68); I² =	= 0%				
Test for overall effect:	Z = 2.76 (P = 0.0	06)					
5.1.3 ALL								
Liang Ting 2009	18	48	5	35	13.5%	3.60 [1.18, 10.95]	2009	
Yan-Hua Xiao 2011	22	57	14	93	24.5%	3.55 [1.63, 7.73]	2011	 − ∎ −
Jie Xiaomei 2012	5	13	4	20	7.3%	2.50 [0.52, 11.96]	2012	
Subtotal (95% CI)		118		148	45.2%	3.39 [1.88, 6.13]		
Total events	45		23					
Heterogeneity: Chi ² =	0.17, df=	2 (P =	0.92); I² =	= 0%				
Test for overall effect:	Z = 4.05 ((P < 0.0	001)					
Total (95% CI)		356		444	100.0%	6.45 [2.88, 14.45	1	•
Total events	197		69					
Heterogeneity: Tau ² =	1.11; Chi	² = 35.9	53, df = 8	(P < 0.	0001); l² :	= 77%		
Test for overall effect:	Z= 4.53 (P < 0.0	0001)	-				0.01 0.1 1 10 100
Test for subaroup diffe	erences:	Chi ^z = :	33.53. df	= 2 (P <	< 0.00001). I ^z = 94.0%		Decreased risk Increased risk

Figure 5. Meta-analyses of aberrantly methylated glioma pathogenesis-related protein 1 gene in leukemia. ALL, acute lymphocytic leukemia; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; M-H, Mantel-Haenszel model; CI, confidence interval; df, degree of freedom.



	Case	•	Contr	ol		Odds Ratio		Odds Ratio
Study or Subgroup	Events	Total	Events	Total	Weight	M-H, Fixed, 95% C	l Year	M-H, Fixed, 95% CI
4.1.1 AML								
Zhang Yan 2010	1	30	1	123	11.3%	4.21 [0.26, 69.27]	2010	
Zhang Hui 2012	21	58	0	31	12.3%	36.12 [2.10, 620.44]	2012	\rightarrow
Subtotal (95% CI)		88		154	23.5%	20.83 [3.01, 143.95]		
Total events	22		1					
Heterogeneity: Chi ² = 1	.40, df =	1 (P = ().24); l ² =	28%				
Test for overall effect: 2	Z = 3.08 (I	P = 0.0	02)					
4.1.2 ALL								
Liu Jianhui 2005	10	26	0	18	10.7%	23.55 [1.28, 433.79]	2005	→
Xu Wen 2005	12	42	0	10	16.8%	8.61 [0.47, 158.37]	2005	→
Wu Chaovang 2008	10	30	0	16	12.7%	16.90 [0.92, 310.32]	2008	
Zhang Yan 2010	10	112	1	123	25.8%	11.96 [1.51, 95.01]	2010	
Yu Gang 2014	10	32	0	30	10.5%	28.47 [1.58, 511.62]	2014	\rightarrow
Subtotal (95% CI)		242		197	76.5%	15.92 [4.87, 52.07]		
Total events	52		1					
Heterogeneity: Chi ² = 0	.47, df =	4 (P = (0.98); l ² =	0%				
Test for overall effect: 2	z = 4.58 (I	P < 0.0	0001)					
Total (95% CI)		330		351	100.0%	17.07 [6.20, 47.02]		•
Total events	74		2					
Heterogeneity: Chi ² = 1	.72, df =	6 (P = (0.94); l ² =	0%				
Test for overall effect: 2	Z = 5.49 (I	P < 0.0	0001)					U.U1 U.1 1 10 100
Test for subaroup diffe	rences: C	hi² = 0.	05. df = 1	(P = 0.1)	.82). I ² = (0%		Decreased lisk Increased lisk

Figure 6. Meta-analyses of aberrantly methylated *p73* in leukemia. ALL, acute lymphocytic leukemia; AML, acute myelogenous leukemia; M-H, Mantel-Haenszel model; CI, confidence interval; df, degree of freedom.

	Case	е	Contr	ol	Odds Ratio		Odds Ratio
Study or Subgroup	Events	Total	Events	Total	M-H, 95% CI	Year	M-H, 95% Cl
2.1.1 Chinese							
Zhang Junli 2000 (ALL)	20	40	0	15	31.00 [1.74, 553.31]	2000	· · · · · · · · · · · · · · · · · · ·
Tan Guangxiao 2001 (AML)	14	20	0	20	91.46 [4.77, 1754.50]	2001	
Yin Yumin 2002 (ALL)	6	15	0	12	17.11 [0.85, 342.74]	2002	
Jiang Junhuang 2002 (AML)	14	18	0	20	132.11 [6.59, 2648.61]	2002	
Jiang Junhuang 2002 (ALL)	19	31	0	20	63.96 [3.54, 1155.34]	2002	
Wang Boxun 2002 (ALL)	11	15	0	12	63.89 [3.09, 1321.68]	2002	
Yang Junzheng 2003 (AML)	9	43	0	20	11.29 [0.62, 204.34]	2003	
Chen Hui 2003 (HapII ~AML)	11	31	0	8	9.54 [0.50, 180.78]	2003	
Chen Hui 2003 (Nrul~AML)	22	31	2	8	7.33 [1.24, 43.41]	2003	→
Chen Hui 2003 (SacII ~AML)	19	31	1	8	11.08 [1.21, 101.68]	2003	→
Yang Junzheng 2003 (ALL)	5	28	0	20	9.60 [0.50, 184.26]	2003	
Zheng Ruiji 2004 (ALL)	12	20	0	20	60.29 [3.20, 1137.79]	2004	│
Song Guoving 2004 (ALL)	5	28	0	20	9.60 [0.50, 184,26]	2004	
Zhu Chuansheng 2005 (ALL)	8	19	0	10	15.52 (0.79, 303.25)	2005	+
Mena Yueshena 2005 (AML)	3	26	0	10	3.13 [0.15, 66,12]	2005	
Meng Yuesheng 2005 (ALL)	2	14	Ō	10	4.20 [0.18, 97, 55]	2005	
Fan Liping 2007 (ALL)	8	24	0	16	17.00 (0.91, 319.22)	2007	<u>↓</u> →
Fan Liping 2007 (AML)	24	58	Ő	16	23.43 [1.34, 409.51]	2007	
Hsiao PC 2008 (AML)	5	6	Ő	8	62.33 [2.13, 1822.63]	2008	│
Hsiao PC 2008 (ALL)	11	13	0	8	78.20 [3.31, 1849.02]	2008	│
Hsian PC 2008 (CML)	1	3	Ő	Ř	10.20 [0.31, 336.93]	2008	
Hsiao PC 2008 (CLL)	. 1	1	õ	8	51.00 [0.70, 3710.31]	2008	+
Xiao Yun 2010 (ALL)	7	17	ň	16	23 57 [1 21 457 36]	2010	→
Xiao Yun 2010 (CLL)	. 1	6	ň	16	9 00 10 32 254 721	2010	
Xiao Yun 2010 (AML)	7	21	ň	16	17 07 10 89 325 59	2010	<u>↓</u>
Xiao Yun 2010 (CML)	. 1	7	ň	16	7 62 [0 27 212 08]	2010	
Subtotal (95% CI)		566	, v	361	19.99 [11.37, 35,17]	2010	•
Total events	246		3				
Heterogeneity: Chi ² = 11 19 df	= 25 (P = 1	n 99) [,] I	²=0%				
Test for overall effect: Z = 10.40) (P < 0.00	001)	- 070				
2.1.2 European							
Deligezer U 2006(p14~AML)	12	24	30	82	1.73 [0.69, 4.34]	2006	
Deligezer U 2006(p14~CLL)	6	12	30	82	1.73 [0.51, 5.86]	2006	
Deligezer U 2006(p14~CML)	7	23	30	82	0.76 [0.28, 2.05]	2006	
Deligezer U 2006(p16~AML)	22	24	73	82	1.36 [0.27, 6.75]	2006	
Deligezer U 2006(p16~CLL)	11	12	73	82	1.36 [0.16, 11.77]	2006	
Deligezer U 2006(p16~CML)	19	23	73	82	0.59 [0.16, 2.11]	2006	-
Cechova H 2012 (p14~AML)	8	13	0	26	81.91 [4.09, 1639.23]	2012	
Cechova H 2012 (p16~AML)	6	13	0	26	45.93 [2.31, 912.04]	2012	
Subtotal (95% CI)		144		544	1.81 [0.80, 4.11]		
Total events	91		309				
Heterogeneity: Tau ² = 0.73; Ch	i ² = 16.78,	df = 7 ((P = 0.02)	; I² = 58%	6		
Test for overall effect: Z = 1.42	(P = 0.16)						
Total (95% CI)		710		905	9.19 [4.93, 17.10]		•
Total events	337		312				
Heterogeneity: Tau ² = 1.63; Ch	i ² = 76.02.	df = 33	(P < 0.00	001); I ² =	57%		
Test for overall effect: Z = 6.99	(P < 0.000	01)					0.05 0.2 1 5 20
Test for subaroup differences:	Chi ² = 20.7	71. df=	1 (P < 0.	00001). I	²= 95.2%		Decreased fisk increased fisk

Figure 7. Meta-analyses of aberrantly methylated *CDKN2A* in Asian and European populations. ALL, acute lymphocytic leukemia; CLL, chronic lymphocytic leukemia; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; M-H, Mantel-Haenszel model; CI, confidence interval; df, degree of freedom.

	Case	•	Contro	ol	Odds Ratio		Odds Ratio		
Study or Subgroup	Events	Total	Events	Total	M-H, 95% Cl	Year	M-H, 95% CI		
2.1.1 Chinese									
Guo Xiuzhi 2000 (AML)	26	31	0	30	293.91 [15.51, 5567.75]	2000			
Chen Fei 2000 (ALL)	5	10	0	10	21.00 [0.97, 453.91]	2000			
Zhu Chuansheng 2001 (ALL)	12	21	0	10	27.63 [1.43, 533.13]	2001			
Tan Guangxiao 2001 (AML)	16	20	0	20	150.33 [7.54, 2997.83]	2001			
Fan Hongtao 2001 (CML)	5	7	0	20	90.20 [3.76, 2166.61]	2001			
Wang Haiyan 2002 (ALL)	7	10	0	7	32.14 [1.40, 736.17]	2002			
Wang Haiyan 2002 (AML)	22	33	0	7	29.35 [1.54, 560.47]	2002			
Shen Zhijian 2002 (AML)	10	25	0	10	14.23 [0.75, 269.96]	2002	+		
Shen Zhijian 2002 (ALL)	6	10	0	10	30.33 [1.39, 660.76]	2002			
Yin Yumin 2003 (ALL)	6	15	0	12	17.11 [0.85, 342.74]	2003			
Yin Yumin 2003 (AML)	17	22	0	12	79.55 [4.02, 1573.39]	2003			
Chen Hui 2003 (AML)	16	31	0	8	18.10 [0.96, 340.68]	2003			
Zheng Ruiji 2004 (ALL)	18	26	0	20	89.24 [4.81, 1655.53]	2004	$ \longrightarrow$		
Tong Hongyan 2004 (ALL)	4	10	0	10	14.54 [0.67, 316.69]	2004	+		
Tong Hongyan 2004 (CML)	5	14	0	10	12.16 [0.59, 250.42]	2004			
Tong Hongyan 2004 (AML)	5	10	0	10	21.00 [0.97, 453.91]	2004			
Zhu Chuansheng 2005 (ALL)	7	19	0	10	12.60 [0.64, 247.56]	2005	+		
Qiao Shukai 2005 (AML)	34	42	0	14	117.71 [6.36, 2176.72]	2005	$ \longrightarrow$		
Qiao Shukai 2005 (ALL)	9	14	0	14	50.09 [2.47, 1014.62]	2005			
Meng Yuesheng 2005 (AML)	24	26	0	10	205.80 [9.08, 4666.41]	2005			
Meng Yuesheng 2005 (ALL)	10	14	0	10	49.00 [2.33, 1028.86]	2005			
Lin Fuan 2012 (ALL)	17	25	0	10	43.24 [2.26, 828.53]	2012	——→		
Wu Dansen 2013 (ALL)	14	14	0	14	841.00 [15.61, 45321.27]	2013	$ \longrightarrow$		
Wu Dansen 2013 (AML)	6	14	0	14	22.18 [1.11, 444.74]	2013			
Subtotal (95% CI)		463		302	42.45 [22.98, 78.42]		•		
Total events	301		0						
Heterogeneity: Chi ² = 10.38, df:	= 23 (P = 0	.99); P	²= 0%						
Test for overall effect: Z = 11.97	(P < 0.000	001)							
2.1.2 European									
Christiansen DH 2003(AML)	19	31	0	6	20.28 [1.05, 392.46]	2003			
Yalcin A 2009 (ALL)	5	6	8	18	6.25 [0.60, 64.86]	2009			
Yalcin A 2009 (AML)	20	26	8	18	4.17 [1.13, 15.33]	2009			
Cechova H 2012 (AML)	13	13	0	26	1431.00 [26.89, 76150.35]	2012			
Subtotal (95% CI)		76		68	16.87 [2.17, 130.95]		-		
Total events	57		16						
Heterogeneity: Tau ² = 2.67; Chi	² = 8.44, di	í= 3 (P	? = 0.04); l	²= 649	•				
Test for overall effect: Z = 2.70 (P = 0.007)								
Total (95% CI)		539		370	30.98 [18.83, 50.97]		•		
Total events	358	223	16						
Heterogeneity Chi ² = 25.96 df	= 27 (P = 1	152) [,] P	 ۳= ۱%						
Test for overall effect $Z = 1351$ ($P < 0.00001$) 0.02 0.1 1 10 50									
Test for subgroup differences: Chi ² = 5.52, df = 1 (P = 0.02), l ² = 81.9%									

Figure 8. Meta-analyses of aberrantly methylated cyclin-dependent kinase 2B gene in Chinese and European populations. ALL, acute lymphocytic leukemia; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; M-H, Mantel-Haenszel model; CI, confidence interval; df, degree of freedom.

Of note, the present meta-analysis had certain limitations. First, the numbers of the studies regarding each gene and leukemia subtype were uneven. For certain leukemia subtypes, only a few studies on certain genes were available. The lack of association of the methylation status of certain genes with several leukemia subtypes may have been due to a lack of statistical power of the respective studies, so that the negative results must be interpreted with caution. Furthermore, a language bias was present, as only studies written in Chinese and English were included.

In conclusion, the present meta-analysis revealed that aberrant DNA methylation of the promoters of 47 genes was associated with leukemia. Further subgroup meta-analysis revealed 5 hypermethylated genes (*CDKN2A*, *CDKN2B*, *ID4*, *GliPR1* and *p73*) in various leukemia subtypes. In addition, a difference in the association of *CDKN2A* and *CDKN2B* hypermethylation with leukemia was identified between Chinese and European populations. The results of the present study may enhance the current understanding of the association of DNA methylation with leukemia in the Chinese population.

Acknowledgements

The present study was supported by grants from National Natural Science Foundation of China (nos. 31100919 and 81371469), Natural Science Foundation of Zhejiang Province (no. LR13H020003), K.C. Wong Magna Fund at Ningbo University and Ningbo Social Development Research Projects (nos. 2010C50019 and 2012C50032).



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