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

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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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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 socio-economic 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 I^2 statistics (19). When there was significant heterogeneity (I^2>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 ≥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).

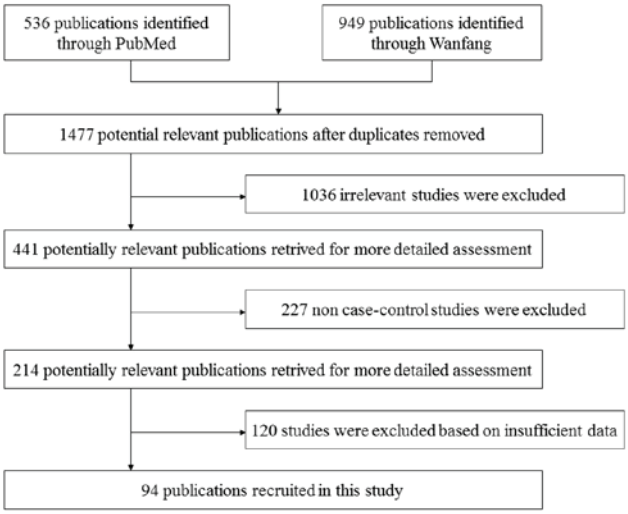

Figure 1. Flow diagram of the study selection process.

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 included 9 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)
RASSF1A	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)
ZO-1	3	99.65 (18.14-547.54)
RARA	3	3.46 (0.65-18.39)
AR	2	98.28 (5.22-1849.18)
CDH13	2	10.18 (1.71-60.56)
DDIT3	2	41.35 (5.41-316.24)
DKK-1	2	24.28 (3.15-187.26)
EDNRB	2	32.57 (3.92-270.80)
FANCF	2	7.09 (3.71-13.53)
GRAF	2	64.36 (8.67-477.53)
HAGE	2	12.23 (1.66-90.39)
hPER3	2	85.56 (10.95-668.76)
miR-34B	2	74.99 (9.65-582.64)
RAGE-1	2	38.88 (5.25-287.87)
RUNX3	2	11.91 (1.45-97.86)
SFRP1	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)
CDH1	1	33.00 (1.78-610.61)
CEBPZ	1	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)
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 $p15^{INK2B}$)

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

Authors	Year	Gene	Disease	Cases (n)		Controls (n)		Refs.
				Meth	Total	Meth	Total	
Hsiao *et al*	2008	*CDKN2A*	ALL	11	13	0	8	(31)
Hsiao *et al*	2008	*CDKN2A*	CLL	1	1	0	8	(31)
Hsiao *et al*	2008	*CDKN2A*	AML	5	6	0	8	(31)
Hsiao *et al*	2008	*CDKN2A*	CML	1	3	0	8	(31)
Zheng *et al*	2004	*CDKN2A*	ALL	12	20	0	20	(32)
Xiao *et al*	2010	*CDKN2A*	AML	7	21	0	16	(33)
Xiao *et al*	2010	*CDKN2A*	ALL	7	17	0	16	(33)
Xiao *et al*	2010	*CDKN2A*	CML	1	7	0	16	(33)
Xiao *et al*	2010	*CDKN2A*	CLL	1	6	0	16	(33)
Yang *et al*	2003	*CDKN2A*	ALL	5	28	0	20	(34)
Yang *et al*	2003	*CDKN2A*	AML	9	43	0	20	(34)
Song *et al*	2004	*CDKN2A*	ALL	5	28	0	20	(35)
Tan *et al*	2001	*CDKN2A*	AML	14	20	0	20	(36)
Zhu *et al*	2005	*CDKN2A*	ALL	8	19	0	10	(37)
Zhang *et al*	2000	*CDKN2A*	ALL	20	40	0	15	(38)
Fan *et al*	2007	*CDKN2A*	AML	24	58	0	16	(39)
Fan *et al*	2007	*CDKN2A*	ALL	8	24	0	16	(39)
Jiang *et al*	2002	*CDKN2A*	ALL	19	31	0	20	(40)
Jiang *et al*	2002	*CDKN2A*	AML	14	18	0	20	(40)
Meng *et al*	2005	*CDKN2A*	AML	3	26	0	10	(41)
Meng *et al*	2005	*CDKN2A*	ALL	2	14	0	10	(41)
Wang *et al*	2002	*CDKN2A*	ALL	11	15	0	12	(42)
Yin *et al*	2002	*CDKN2A*	ALL	6	15	0	12	(43)
Chen *et al*	2003	*CDKN2A* (*Hap*II)	AML	11	31	0	8	(44)
Chen *et al*	2003	*CDKN2A* (*Nru*I)	AML	22	31	2	8	(44)
Chen *et al*	2003	*CDKN2A* (*Sac*II)	AML	19	31	1	8	(44)
Lin *et al*	2012	*CDKN2B*	ALL	17	25	0	10	(45)
Zheng *et al*	2004	*CDKN2B*	ALL	18	26	0	20	(32)
Chen *et al*	2003	*CDKN2B*	AML	16	31	0	8	(44)
Tan *et al*	2001	*CDKN2B*	AML	16	20	0	20	(36)
Zhu *et al*	2001	*CDKN2B*	ALL	12	21	0	10	(46)
Zhu *et al*	2005	*CDKN2B*	ALL	7	19	0	10	(37)
Shen *et al*	2002	*CDKN2B*	ALL	6	10	0	10	(47)
Shen *et al*	2002	*CDKN2B*	AML	10	25	0	10	(47)
Fan *et al*	2001	*CDKN2B*	CML	5	7	0	20	(48)
Tong *et al*	2004	*CDKN2B*	AML	5	10	0	10	(49)
Tong *et al*	2004	*CDKN2B*	ALL	4	10	0	10	(49)
Tong *et al*	2004	*CDKN2B*	CML	5	14	0	10	(49)
Guo *et al*	2000	*CDKN2B*	AML	26	31	0	30	(50)
Qiao *et al*	2005	*CDKN2B*	AML	34	42	0	14	(51)
Qiao *et al*	2005	*CDKN2B*	ALL	9	14	0	14	(51)
Chen *et al*	2000	*CDKN2B*	ALL	5	10	0	10	(52)
Yin *et al*	2003	*CDKN2B*	ALL	6	15	0	12	(53)
Yin *et al*	2003	*CDKN2B*	AML	17	22	0	12	(53)
Meng *et al*	2005	*CDKN2B*	AML	24	26	0	10	(41)
Meng *et al*	2005	*CDKN2B*	ALL	10	14	0	10	(41)
Wu *et al*	2013	*CDKN2B*	ALL	14	14	0	14	(54)
Wu *et al*	2013	*CDKN2B*	AML	6	14	0	14	(54)
Wang *et al*	2002	*CDKN2B*	ALL	7	10	0	7	(55)
Wang *et al*	2002	*CDKN2B*	AML	22	33	0	7	(55)

Table II. Continued.

Authors	Year	Gene	Disease	Cases (n)		Controls (n)		Refs.
				Meth	Total	Meth	Total	
Zhao *et al*	2008	ID4	AML	15	32	0	18	(56)
Wang *et al*	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 *et al*	2005	*ID4*	AML	21	25	0	49	(60)
Zhao *et al*	2005	*ID4*	CML	2	4	0	49	(60)
Zhao *et al*	2005	*ID4*	ALL	12	14	0	49	(60)
Zhao *et al*	2005	*ID4*	CLL	3	3	0	49	(60)
Xiao *et al*	2011	*GLIPR1*	AML	58	70	14	93	(61)
Xiao *et al*	2011	*GLIPR1*	CML	11	40	14	93	(61)
Xiao *et al*	2011	*GLIPR1*	ALL	22	57	14	93	(61)
Liang *et al*	2009	*GLIPR1*	AML	44	54	5	35	(62)
Liang *et al*	2009	*GLIPR1*	CML	11	40	5	35	(62)
Liang *et al*	2009	*GLIPR1*	ALL	18	48	5	35	(62)
Jie *et al*	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 *et al*	2010	*p73*	AML	1	30	1	123	(63)
Zhang *et al*	2010	*p73*	ALL	10	112	1	123	(63)
Zhang *et al*	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 *et al*	2001	*CT*	CLL	1	3	0	30	(70)
Tang *et al*	2001	*CT*	CML	8	10	0	30	(70)
Tang *et al*	2001	*CT*	ALL	12	14	0	30	(70)
Wang *et al*	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 *et al*	2009	*DAPK*	AML	3	60	0	17	(74)
Zhao *et al*	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 *et al*	2003	*PRB*	ALL	6	11	0	10	(78)
Zhang *et al*	2003	*PRB*	CLL	6	8	0	10	(78)
Zhang *et al*	2003	*PRB*	AML	9	15	0	10	(78)
Zhang *et al*	2003	*PRB*	CML	6	10	0	10	(78)
Shi *et al*	2011	*SFRP5*	AML	10	99	1	70	(79)
Wang *et al*	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.

Authors	Year	Gene	Disease	Cases (n)		Controls (n)		Refs.
				Meth	Total	Meth	Total	
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 *et al*	2003	*PRA*	ALL	7	11	0	10	(78)
Zhang *et al*	2003	*PRA*	CLL	5	8	0	10	(78)
Zhang *et al*	2003	*PRA*	AML	10	15	0	10	(78)
Zhang *et al*	2003	*PRA*	CML	7	10	0	10	(78)
Chen *et al*	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 *et al*	2011	*SFRP2*	CML	25	38	0	13	(84)
Shi *et al*	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 *et al*	2004	*LRP15*	AML	37	53	0	9	(85)
Dou *et al*	2004	*LRP15*	ALL	15	20	0	9	(85)
Dou *et al*	2004	*LRP15*	CLL	1	2	0	9	(85)
Yao *et al*	2010	*RIZ1*	AML	11	37	0	15	(86)
Cai *et al*	2012	*RIZ1*	ALL	15	64	0	9	(87)
Cai *et al*	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 *et al*	2012	*WT1*	AML	8	23	15	20	(59)
Jie *et al*	2012	*WT1*	ALL	4	13	15	20	(59)
Jie *et al*	2012	*WT1*	CML	7	11	15	20	(59)
Dou *et al*	2009	*ZO-1*	Leukemia	7	10	0	10	(89)
Wang *et al*	2008	*ZO-1*	AML	32	52	0	40	(90)
Wang *et al*	2008	*ZO-1*	ALL	17	29	0	40	(90)
Chim *et al*	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 *et al*	2007	*AR*	ALL	4	4	0	3	(92)
Wang *et al*	2007	*AR*	AML	11	11	0	3	(92)
Wang *et al*	2009	*CDH13*	CML	4	8	0	5	(93)
Liu *et al*	2013	*CDH13*	AML	23	44	1	10	(94)
Wang *et al*	2009	*DDIT3*	AML	62	133	0	16	(95)
Wang *et al*	2009	*DDIT3*	CML	39	59	0	16	(95)
Zhu *et al*	2012	*DKK-1*	ALL	14	34	0	20	(96)
Zhu *et al*	2012	*DKK-1*	AML	10	31	0	20	(96)
Yuan *et al*	2010	*EDNRB*	AML	15	22	0	8	(97)
Yuan *et al*	2010	*EDNRB*	ALL	11	17	0	8	(97)
Yu *et al*	2008	*FANCF*	AML	41	58	7	20	(98)
Deng *et al*	2009	*FANCF*	AML	85	111	11	42	(99)
Qian *et al*	2010	*GRAF*	AML	87	132	0	20	(100)
Qian *et al*	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.

Authors	Year	Gene	Disease	Cases (n)		Controls (n)		Refs.
				Meth	Total	Meth	Total	
Wang *et al*	2011	*hPER3*	AML	116	206	0	40	(103)
Wang *et al*	2013	*miR-34B*	ALL	24	31	0	23	(104)
Wang *et al*	2013	*miR-34B*	AML	8	19	0	23	(104)
Chai *et al*	2013	*RAGE-1*	AML	52	121	0	25	(105)
Chai *et al*	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 *et al*	2011	*WIF1*	AML	11	34	0	15	(108)
Wang *et al*	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 *et al*	2011	*CEBPZ*	AML	62	133	0	20	(111)
Guan *et al*	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 *et al*	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 *et al*	2013	*PRAME*	CML	28	55	0	20	(118)
Yan *et al*	2012	*PRDX2*	AML	17	55	0	40	(119)
Yang *et al*	2007	*PTEN*	ALL	5	22	0	25	(120)
Du *et al*	2013	*RIL*	AML	50	60	0	20	(121)
Jiao *et al*	2013	*SALL4*	AML	9	45	0	20	(122)
Zhuang *et al*	2011	*SOCS-1*	AML	15	24	0	10	(123)
Deng *et al*	2011	*WNT5A*	Leukemia	47	68	0	27	(124)
Zhang *et al*	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 G1 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

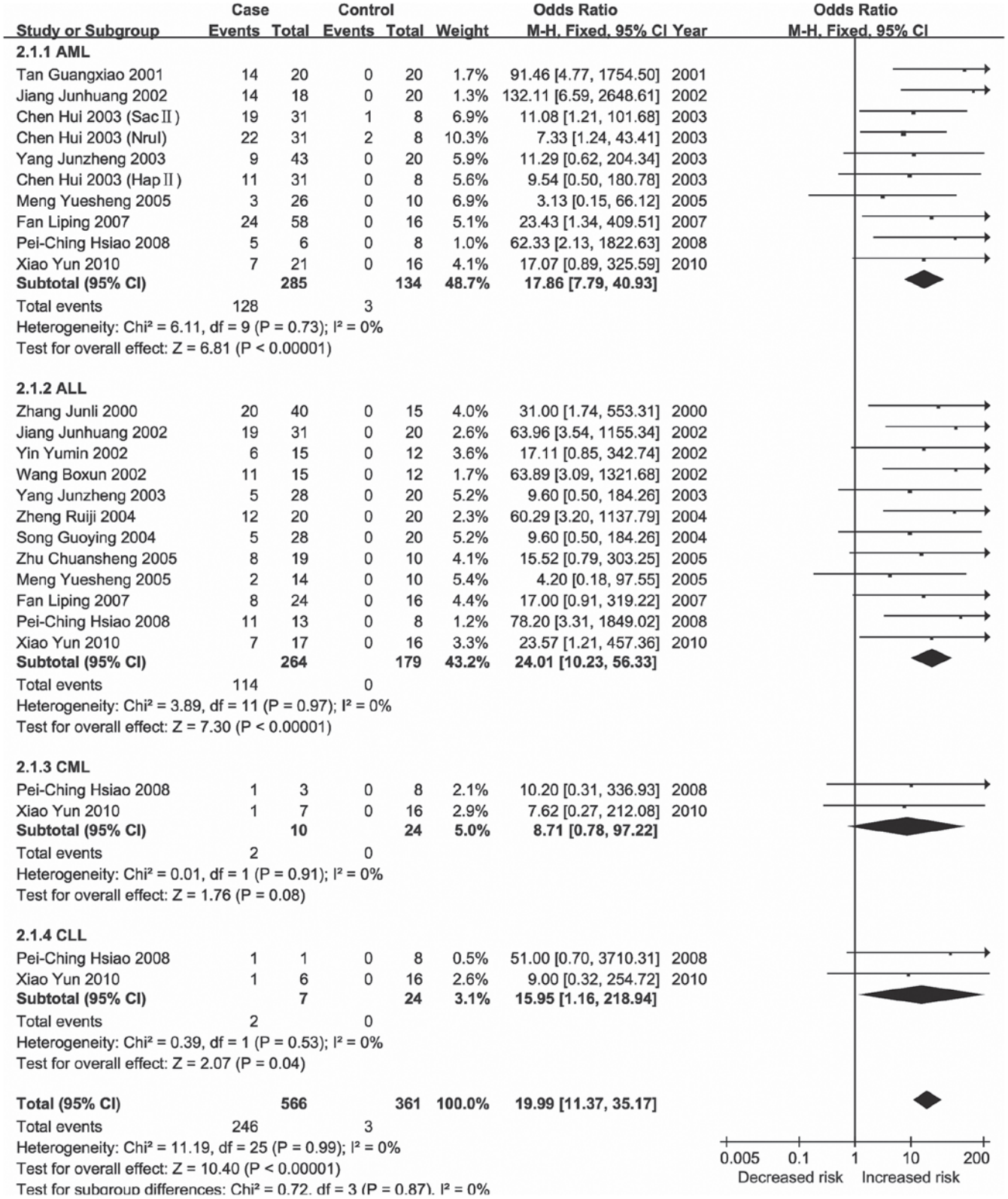

Figure 2. Meta-analyses of aberrantly methylated cyclin-dependent kinase 2A gene in leukemia. ALL, acute lymphocytic leukemia; CLL, chronic lymphocytic leukemia; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; M-H, Mantel-Haenszel model; CI, confidence interval; df, degree of freedom.

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*

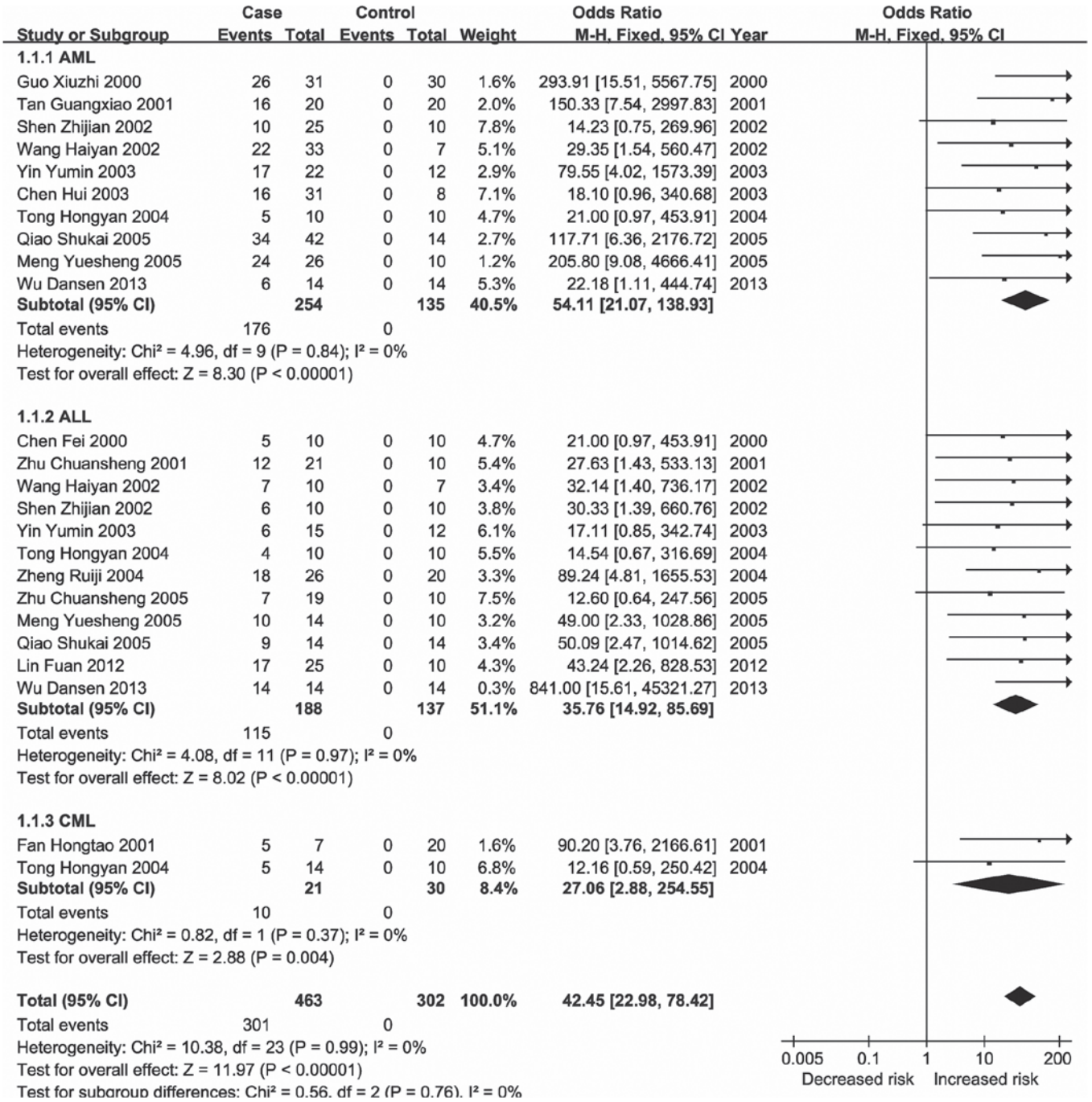

Figure 3. Meta-analyses of aberrantly methylated cyclin-dependent kinase 2B gene in leukemia. ALL, acute lymphocytic leukemia; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; M-H, Mantel-Haenszel model; CI, confidence interval; df, degree of freedom.

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.

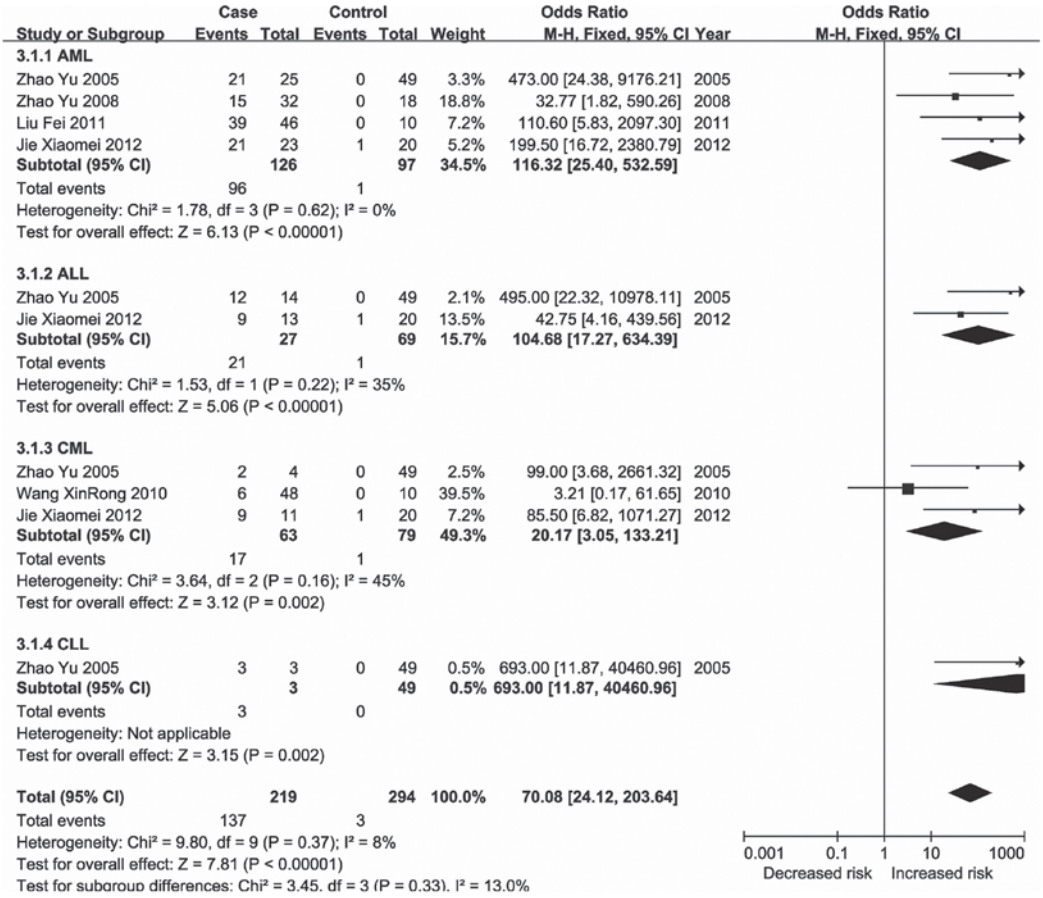

Figure 4. Meta-analyses of aberrantly methylated DNA-binding protein inhibitor-4 gene in leukemia. ALL, acute lymphocytic leukemia; CLL, chronic lymphocytic leukemia; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; M-H, Mantel-Haenszel model; CI, confidence interval; df, degree of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

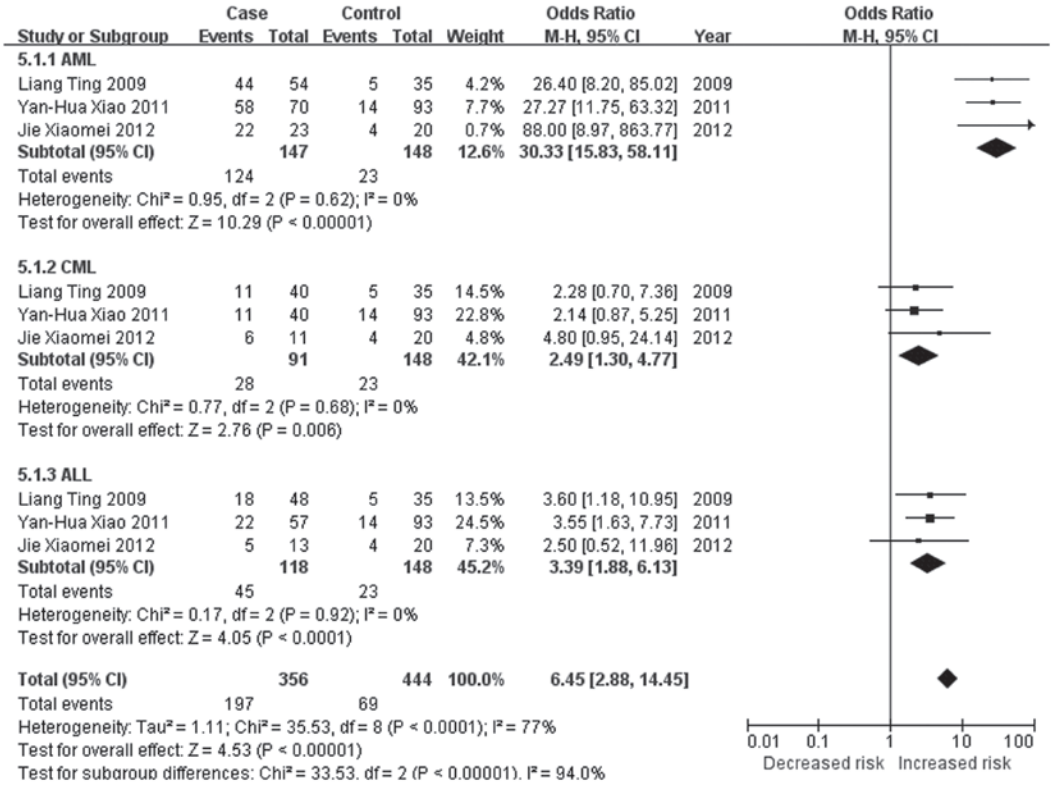

Figure 5. Meta-analyses of aberrantly methylated glioma pathogenesis-related protein 1 gene in leukemia. ALL, acute lymphocytic leukemia; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; M-H, Mantel-Haenszel model; CI, confidence interval; df, degree of freedom.

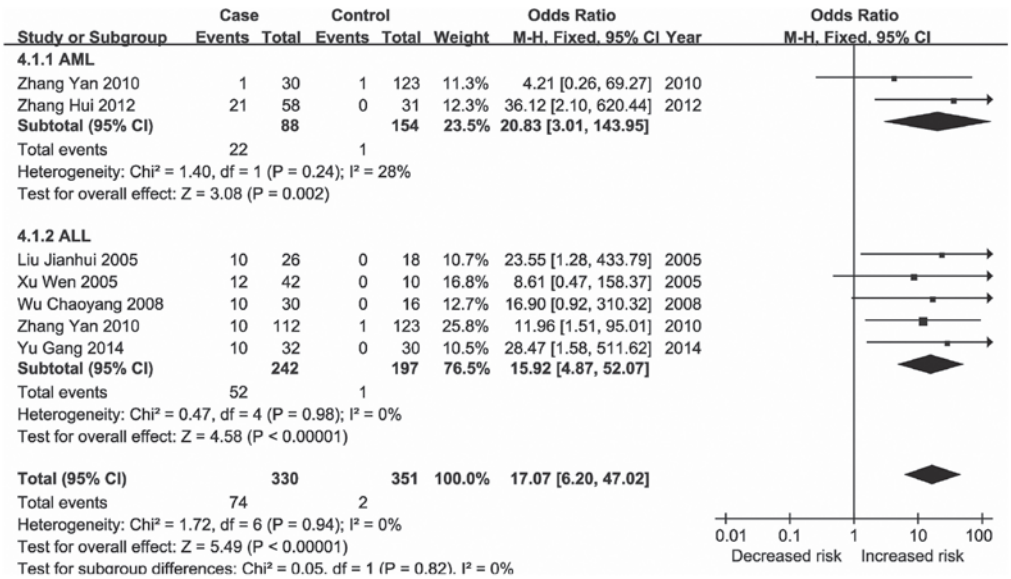

Figure 6. Meta-analyses of aberrantly methylated *p73* in leukemia. ALL, acute lymphocytic leukemia; AML, acute myelogenous leukemia; M-H, Mantel-Haenszel model; CI, confidence interval; df, degree of freedom.

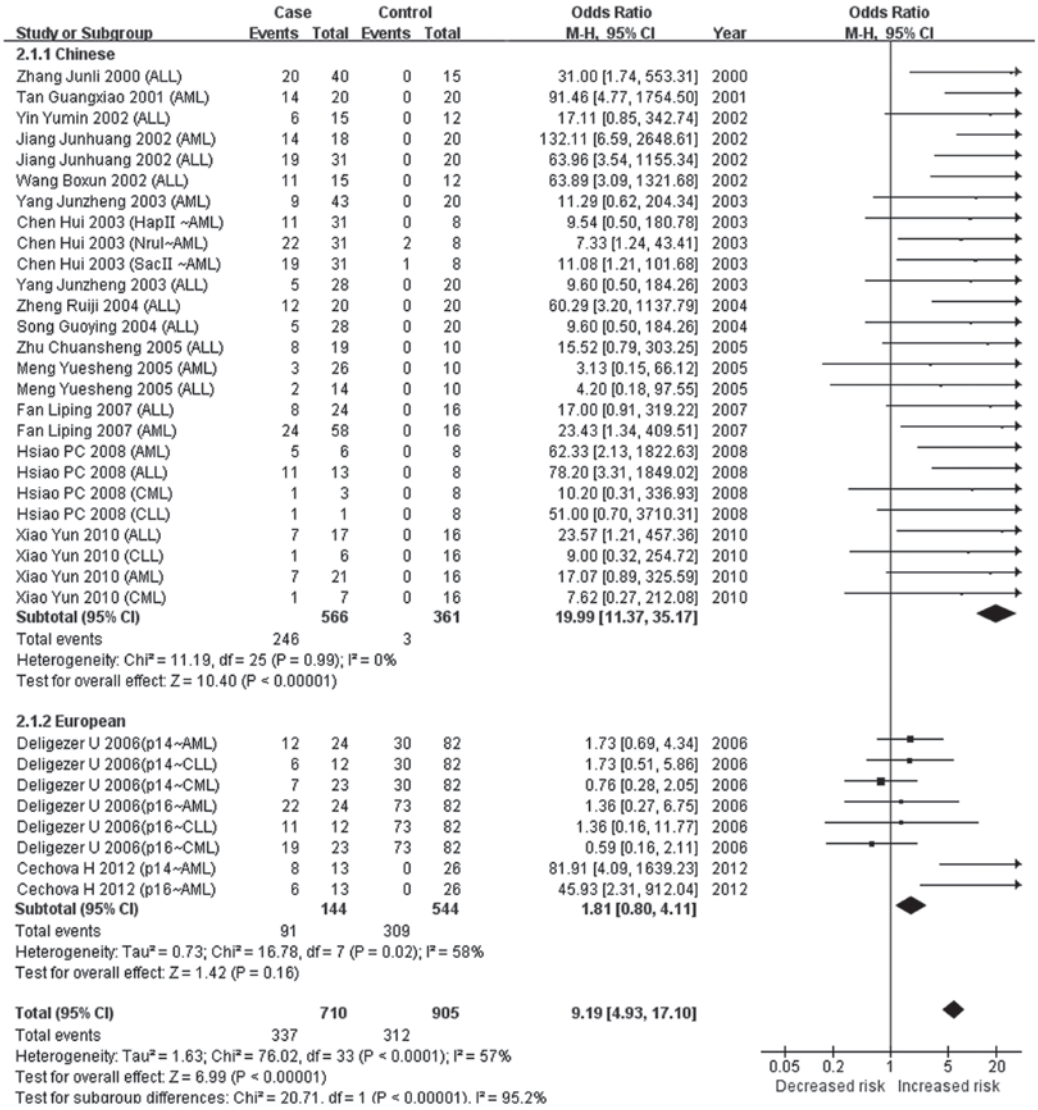

Figure 7. Meta-analyses of aberrantly methylated *CDKN2A* in Asian and European populations. ALL, acute lymphocytic leukemia; CLL, chronic lymphocytic leukemia; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; M-H, Mantel-Haenszel model; CI, confidence interval; df, degree of freedom.

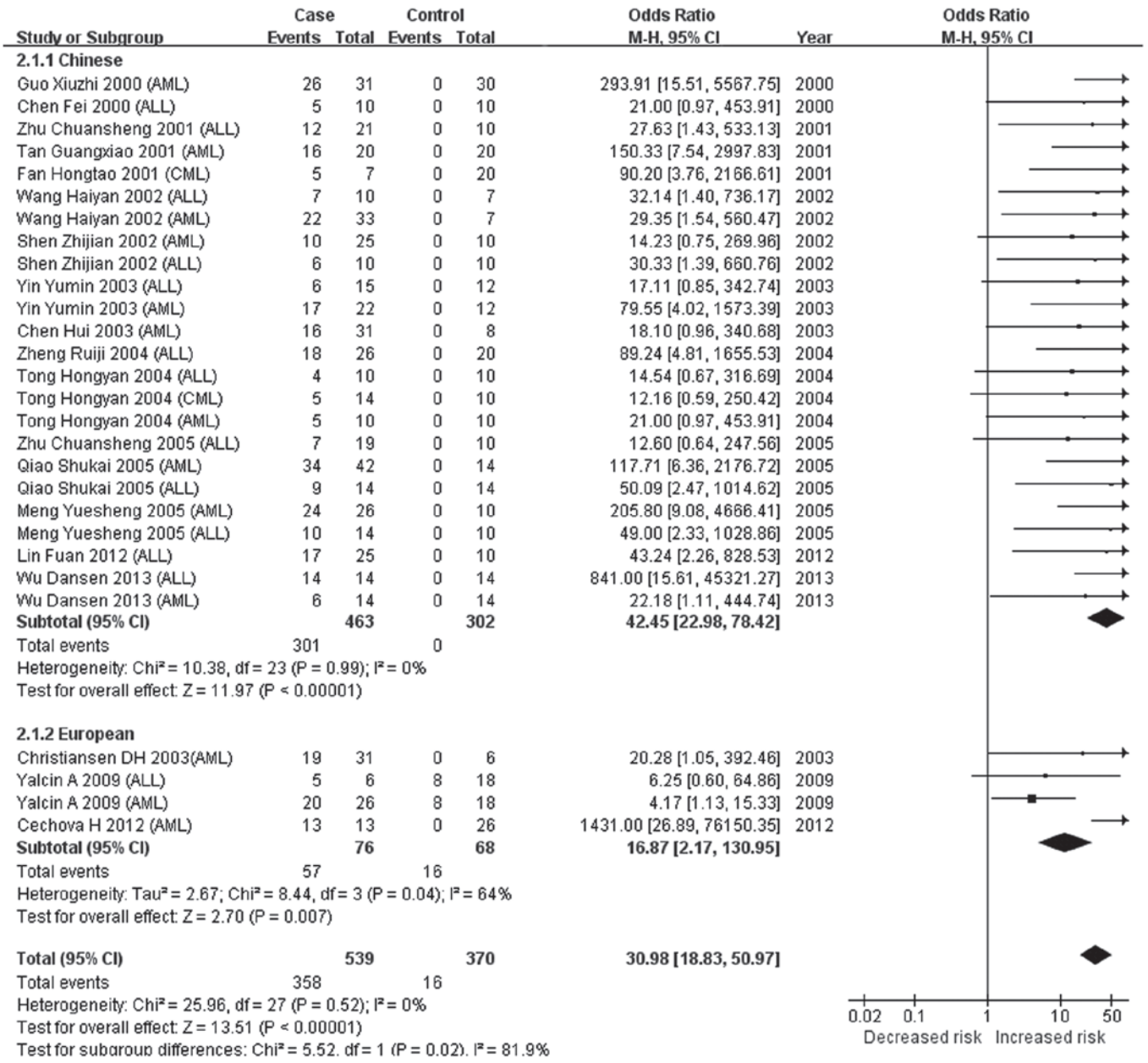

Figure 8. Meta-analyses of aberrantly methylated cyclin-dependent kinase 2B gene in Chinese and European populations. ALL, acute lymphocytic leukemia; AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; M-H, Mantel-Haenszel model; CI, confidence interval; df, degree of freedom.

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.

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