Clinical relevance of the multidrug resistance-associated protein 1 gene in non-small cell lung cancer: A systematic review and meta-analysis

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Abstract. The multidrug resistance-associated protein 1 (MRP1) gene has been found to be consistently overexpressed in the majority of patients with non-small cell lung cancer (NSCLC). MRP1 is known for its ability to actively decrease intracellular drug concentration, limiting the efficacy of cancer chemotherapy; however, data on the clinical relevance of MRP1 is inconclusive. In the present meta-analysis, all available published data were combined to provide an updated view on the clinicopathological relevance of MRP1 in patients with NSCLC. A systematic search was conducted to obtain relevant studies published in English, Chinese and Japanese databases. All data from patients with NSCLC who underwent testing for MRP1, by either immunohistochemistry or reverse transcription-polymerase chain reaction, were extracted and combined for further analysis. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for each selected study, with either the fixed-effects model or the random-effects model where appropriate. The quality of methodology, heterogeneities and publication bias of the included articles were also analyzed. A total of 36 clinical studies involving 3,278 patients were included in the study. It was found that the increased expression of the MRP1 gene was associated with the following subgroups of patients: Non-smokers vs. smokers (OR, 2.54; 95% CI, 1.17-5.54; P=0.019); adenocarcinoma vs. squamous cell carcinoma (OR, 1.58; 95% CI, 1.16-2.17; P=0.004); clinical stage III-IV vs. stage I-II (OR, 1.36; 95% CI, 1.11-1.66; P=0.003); lymph node metastases (OR, 1.32; 95% CI, 1.09-1.61; P=0.005); poor response to chemotherapy (OR, 0.41; 95% CI, 0.23-0.72; P=0.002) and reduced 3-year survival rate (OR, 0.40; 95% CI, 0.23-0.68; P=0.001). In conclusion, the findings from this study suggest that increase in MRP1 gene expression is associated with being a non-smoker, adenocarcinoma, advanced clinical stages and a poor response to chemotherapy in patients with NSCLC. The results from the most extensive and updated data on MRP1 support the requirement for continued investigation into the potential use of MRP1 as a biomarker/clinical indicator for NSCLC.

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Introduction

Lung cancer is the most commonly diagnosed cancer worldwide, responsible for 19.4% of all cancer-related mortalities (1). Non-small cell lung cancer (NSCLC) and SCLC are the main subtypes of lung cancer, with NSCLC representing ~85% of cases (2). While the incidence of lung cancer in the US has been declining since 2005 (1) due to decreased smoking rates (3), the incidence in China for the same period has been increasing, particularly in the female population and the younger generation (4,5). Smoking and air pollution have been reported to be mainly responsible for the increase in lung cancer incidence and mortalities in China (5). The majority of patients with lung cancer are usually diagnosed at an advanced stage, leaving limited treatment options (3,6). As a result, the prognosis of lung cancer patients remains poor, with a 5-year overall survival rate as low as 15%, despite progress being made in the field of NSCLC (7). A poor treatment outcome has been associated with the multidrug resistance-associated protein 1 (MRP1)

gene, which is commonly overexpressed in NSCLC tissues and may limit the efficacy of chemotherapy (8).

MRP1 was first identified and cloned in the anthracycline-selected human small-cell lung carcinoma cell line, H69AR (8). MRP1 is a member of the subfamily C of ATP-binding cassette (ABC) transporters, and is hence also known as ABCC1 (9). Distributed throughout a variety of normal human tissues, MRP1 is present in organs such as the lungs, spleen, testes, kidneys, thyroid, bladder and adrenal glands (9,10). In normal cells, MRP1 mediates the efflux of endogenous metabolites, including glutathione, cysteinyl leukotriene C4, nitric oxides, lipid-derived signaling molecules and antioxidants (11,12). Overexpression of MRP1 is a common phenomenon in various cancer tissues, reducing cytotoxicity and the efficacy of antineoplastic agents by boosting the efflux of the drugs, including cisplatin, vinorelbine and gemcitabine, resulting in shorter tumor-free survival and overall survival (OS) times in patients with NSCLC (9,13,14).

Although MRP1 has been known about and studied for more than two decades, and found to be consistently overexpressed in the majority of patients with NSCLC, the clinical relevance of MRP1 expression in these patients remains inconclusive (15). This is not unexpected, as it has been a challenge to define precisely the relevance of MRP1 in clinical drug resistance in patients with cancer (12). As aforementioned, MRP1 is an ABC membrane transport protein implicated in clinical drug resistance, and is capable of actively decreasing the intracellular drug concentration in the cells. Thus, MRP1 expression may affect the clinical outcome of chemotherapy for NSCLC. Indeed, certain studies showed that MRP1 expression was a significant indicator of a poor response to chemotherapy and poor OS in NSCLC (16). However, another study showed that the expression of MRP1 had no correlation with OS or response to chemotherapy in NSCLC, and no significant correlations between MRP1 expression and the clinicopathological parameters of NSCLC were found (15). Therefore there is a requirement to clarify the clinical relevance of MRP1 in NSCLC patients.

The present meta-analysis aimed to systemically investigate the epidemiological and clinicopathological implications associated with increased MRP1 gene in patients suffering from NSCLC by pooling all available published data. Findings from this study will advance our current understanding of the function of MRP1, and in particular, provide novel insight into the clinical significance of the MRP1 gene in NSCLC.

Materials and methods

Article search strategy. Using the terms 'non-small cell lung cancer', 'NSCLC', 'multidrug resistance-associated protein' and 'MRP', a comprehensive search was conducted of articles published until January 2018 from English, Chinese and Japanese databases, including MEDLINE (PubMed, https://www.ncbi.nlm.nih.gov/pubmed), Embase (https://www.elsevier.com/en-in/solutions/embase-biomedical-research), ISI Web of Science (https://clarivate.com/products/web-of-science), Cochrane Library (http://www.cochranelibrary.com), Google Scholar (https://scholar.google.com), China National Knowledge Infrastructure (http://www.cnki.net), VIP Database (http://en.cqvip.com), China Biomedical Literature Database (http://www.sinomed.ac.cn), Wanfang Database (http://www.wanfangdata.com.cn), Medical*Online-E (http://mol.medicalonline.jp/en) and CiNii (https://ci.nii.ac.jp). The search strategy had neither year nor language restrictions. The references of included articles were manually retrieved to find potentially relevant studies. In addition, potentially eligible studies were also identified by reading the meta-analyses and review articles that emerged from the search. Gray literature publications were not searched due to limited resources. The meta-analysis was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses guidelines (17).

Article selection criteria. Two independent researchers screened all the identified articles and selected those that met the following inclusion criteria: Clinical studies involving patients with a confirmed diagnosis of NSCLC and with lung tissue specimens tested for MRP1 by either immunohistochemistry or reverse transcription-polymerase chain reaction (RT-PCR). Moreover, the numbers of MRP1(+) patients against the total number of patients were recorded. The following categories of articles were excluded from the meta-analysis: i) Studies of non-NSCLC carcinomas; ii) lung tissue specimens not tested or analyzed by immunohistochemistry or RT-PCR; iii) animal studies, cell line experiments, case reports, meta-analyses and systematic reviews; and iv) duplicate studies. When overlapping studies were published by the same author(s), the most informative or the most updated studies were selected. Disputes of selection were resolved following discussion with the senior investigators.

Quality assessment. The Newcastle-Ottawa Scale (NOS) was adopted to assess the methodology quality of the 36 studies included in this meta-analysis (18). Since standard criteria for the NOS have not been established, the star system of the NOS (range, 0 to 9 stars) was used for all assessments, with a score of 7 or more stars considered as high quality.

Statistical analysis. All statistical analyses were performed using the Stata software, version 12.0 (StataCorp LP, College Station, TX, USA). The pooled odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to assess the strength of the association between increased MRP1 gene expression and the clinicopathological features of the patients with NSCLC. The significance of the pooled ORs was determined by the Z-test, with $P<0.05$ considered as statistically significant. The degree of heterogeneity between studies was assessed using Cochran's Q-statistic and the I^2 test (variation in OR attribuTable to heterogeneity). An I^2 value of 0% indicated no observed heterogeneity, with larger I^2 values indicating increased heterogeneity; $P<0.10$ was regarded as indicating a statistically significant difference. When statistical heterogeneity existed, the random-effects model was conducted, otherwise the fixed-effects model was applied (19).

Sensitivity analysis. To test the robustness of the results in this meta-analysis, a sensitivity analysis was performed using the one-at-a-time method, which omits one study at a time with repeat meta-analysis to reveal the influence of the individual data sets to the pooled ORs.

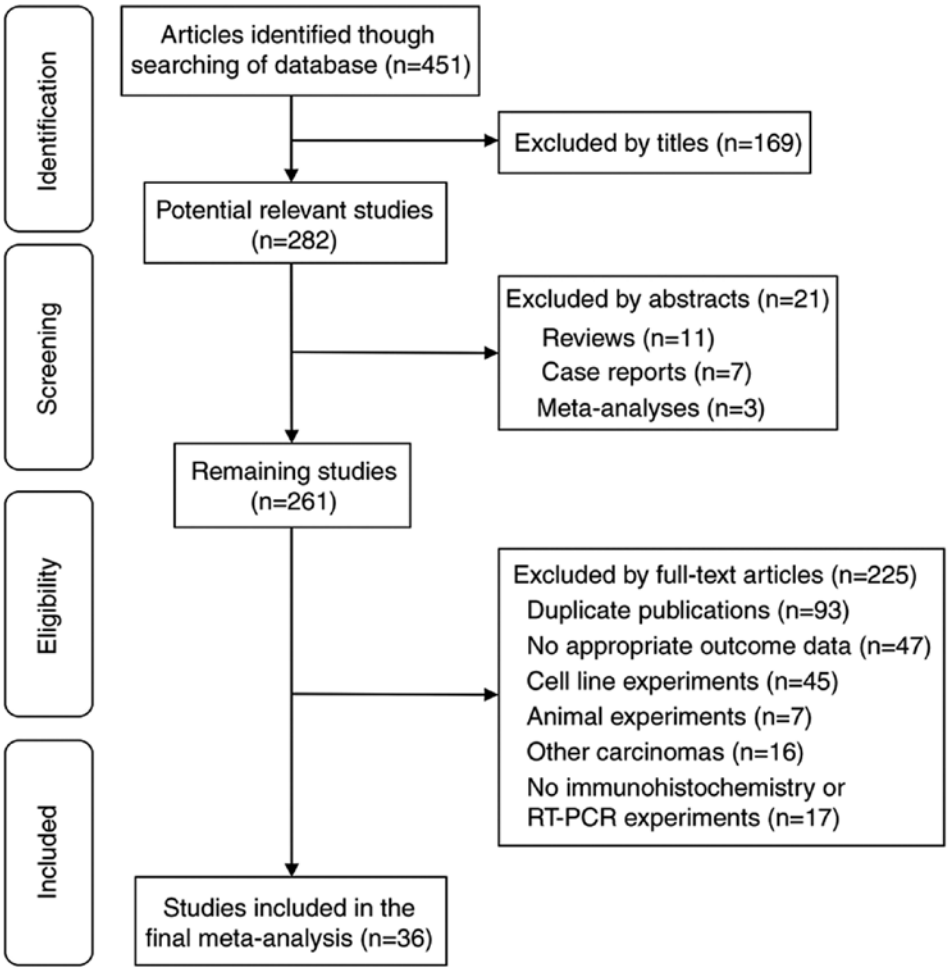

Figure 1. Flow diagram for the systematic review method (Preferred Reporting Items for Systematic Reviews and Meta-Analyses method).

Publication bias. Publication bias was estimated with visual and statistically significant asymmetry using the funnel plot. Begg's funnel plot and Egger's test were employed to assess the potential publication bias in all studies. Publication bias was considered to be present when Begg's funnel plot was asymmetric or when P<0.05 using Egger's test.

Results

A total of 36 clinical studies, involving 3,278 patients, were included in the present meta-analysis, with 2,259 (68.9%) of the total cohort being Asian (20-55). Fig. 1 outlines the results of the selection criteria and the search strategies. Table I summarizes the baseline characteristics of the included studies. Table II highlights the quality of publications included in the study.

The outcomes of meta-analysis and statistical analysis are summarized in Table III. Increased expression of the MRP1 gene was associated with the following subgroups: Non-smokers vs. smokers (OR, 2.54; 95% CI, 1.17-5.54; P=0.019); patients with adenocarcinoma vs. those with squamous cell carcinoma (OR, 1.58; 95% CI, 1.16-2.17; P=0.004); and patients at clinical stage III-IV vs. those at clinical stage I-II (OR, 1.36; 95% CI, 1.11-1.66; P=0.003) (Fig. 2).

Increased expression of the MRP1 gene was also associated with the following subgroups: NSCLC tissue vs. normal pulmonary tissue adjacent to NSCLC (OR, 5.54; 95% CI, 3.69-8.32; P<0.001) (Fig. 3); increased tendency of lymph node metastases (OR, 1.32; 95% CI, 1.09-1.61; P=0.005) (Fig. 4); compromised response to chemotherapy (OR, 0.41; 95% CI, 0.23-0.72; P=0.002); and decreased 3-year survival rate (OR, 0.40; 95% CI, 0.23-0.68; P=0.001) (Fig. 5).

There were no significant differences in MRP1 gene expression in the following subgroups: Male vs. female, patients ≥60 years old vs. <60 years old, NSCLC cohorts with cytological grades 1 and 2 vs. cytological grades 3 and 4, and 1-year survival rate (Figs. 3-5).

Since the heterogeneity among studies included in the analysis of the associations of MRP1 expression with pathological type and 3-year survival rate were significantly high (I^2 value of 59.2 and 62.5%, respectively; Table III), a sensitivity analysis was conducted to assess the stability of the results. The analysis showed that the significance of the results was not affected by any single study, which is visually depicted in Fig. 6, indicating the statistical robustness of the results.

As shown in Fig. 7, the Begg's funnel plots were symmetrical, indicating no evidence of publication bias in the study. The P-value of Egger's test for publication bias was >0.05.

Table I. Characteristics of the 36 studies included in the meta-analysis.

First author/s	Age, years		Sex		Smoking status		Histological origin		Pathological type		Cytological grade		Clinical stage		Lymph node metastasis		Response to chemotherapy		1-year survival rate		3-year survival rate		(Refs.)
	<60	≥60	M	F	S	NS	NT	AT	AD	SQ	G1-G2	G3-G4	III-VI	I-II	M(+)	M(-)	R(+)	NR(-)	MRP1 (+)	MRP1 (-)	MRP1 (+)	MRP1 (-)	
Rybárová *et al*									41/56	44/58	48/59	48/76	19/26	78/109									(20)
Xu *et al*	30/69	36/83	54/127	12/25					25/37	32/97			17/53	49/99	32/78	34/74			50/64	67/82	23/64	45/82	(21)
Chen *et al*			30/53	18/39			54/92	3/20	11/20	21/51	35/56	13/36	23/39	25/53	30/55	18/37			53/54	38/38	19/54	26/38	(22)
Qu *et al*							46/55	8/23	17/24	29/31													(23)
Li *et al*																	5/17	18/29	18/23	20/23	8/23	13/23	(24)
Xie *et al*							23/93	3/23	6/24	17/69	1/37	2/73											(25)
Sun *et al*									11/19	25/41	24/43	6/17	27/51	3/9	15/27	15/33							(26)
Liu *et al*									44/60	24/52	44/57	24/55	39/62	29/50									(27)
Liu *et al*									36/45	22/41	39/49	19/37	52/77	6/9									(28)
Wang *et al*																			16/29	36/44	5/29	21/44	(29)
Wang *et al*																	11/36	18/33					(30)
Filipits *et al*	120/236	101/203	301/637	63/145					109/250	208/435	94/198	270/584	266/511	98/271	218/416	146/366			312/364	360/418	182/364	220/418	(31)
Wang *et al*	23/36	25/30	33/50	15/16	24/37	24/29			27/30	21/36			39/51	9/15									(32)
Zhang *et al*							31/36	9/12															(33)
Zuo *et al*							28/43	2/15	11/15	12/18	17/25	11/18	10/14	18/29	9/14	19/29							(34)
Sun *et al*							31/78	0/15	24/45	7/28	19/51	12/27	8/19	23/59	15/35	16/43							(35)
Zhang *et al*									7/27	13/36													(36)
Li *et al*	61/75	30/38							52/61	30/39	73/90	18/23	8/12	66/78	30/37	44/53							(37)
Li *et al*									7/52	3/33			6/56	5/42			1/16	4/31					(38)
Hao *et al*			49/63	22/29					28/40	43/52	39/57	22/25			35/43	36/49							(39)
Yoh *et al*			38/49	18/23	41/54	15/18											19/25	37/47					(40)
Guo *et al*			29/46	10/12	26/43	13/15			21/22	18/36	31/42	8/16	12/17	26/41	10/19	29/39							(41)
Li *et al*									11/17	8/25	16/35	3/7	5/11	14/31					14/19	19/23	1/19	7/23	(42)
Xia *et al*									21/29	12/18					19/27	14/20							(43)
Peng *et al*																	4/14	10/17					(44)
Huo *et al*									14/19	17/26													(45)
Han *et al*									11/21	8/21			9/23	11/25									(46)
Yang *et al*							13/30	8/30															(47)
Rui *et al*									8/12	7/11													(48)
Wang *et al*							24/45	5/45											7/9	10/11	0/2	3/5	(49)
Wang *et al*							49/66	1/5			32/46	17/22	29/38	7/11									(50)
Xu *et al*							23/32	1/10	13/17	10/15			9/15	14/17	15/23	6/9							(51)
Peng *et al*							50/92	5/16	32/49	18/43	32/64	18/28			33/51	17/41							(51)
Zhan *et al*									28/48	30/42									31/48	19/25	12/48	12/25	(53)
Wright *et al*			52/60	38/42					50/55	38/45	60/67	27/30	12/12	69/79	35/39	55/63							(54)
Xu *et al*									23/30	15/25					28/45	14/16							(55)
Total	234/416	192/354	586/1085	196/331	91/134	52/62	372/662	45/214	688/1124	732/1424	604/976	518/1074	590/1087	550/1027	524/909	463/872	40/108	87/157	501/610	569/664	250/603	347/658	

MRP1, multidrug resistance-associated protein 1; M, male; F, female; S, smoker; NS, non-smoker; NT, NSCLC tumor tissue; AT, adjacent non-cancerous tissue; AD, adenocarcinoma; SQ, squamous cell carcinoma; G1-G2, grades 1 and 2; G3-G4, grades 3 and 4; M(+), positive for lymph node metastasis; M(-), negative for lymph node metastasis; R(+), response to chemotherapy; NR(-), non-response to chemotherapy; MRP1, multidrug resistance-associated protein 1; MRP1(+), elevated expression of MRP1; MRP1(-), low expression of MRP1.

Table II. Methodology of quality assessment of studies included in the final analysis.

First author/s	Adequate definition of patient cases	Representativeness of patient cases	Selection of controls	Definition of controls	Control for important factor or additional factor	Ascertainment of exposure (blinding)	Same method of ascertainment for participants	Non-response rate	Total score[a]	(Refs.)
Rybárová *et al*	1	1	1	1	1	1	1	0	7	(20)
Xu *et al*	1	1	1	1	1	1	1	0	7	(21)
Chen *et al*	1	1	1	1	1	1	1	0	7	(22)
Qu *et al*	1	1	0	1	1	1	1	0	6	(23)
Li *et al*	1	0	0	1	1	1	1	1	6	(24)
Xie *et al*	1	1	0	1	1	1	1	0	6	(25)
Sun *et al*	1	1	0	1	1	1	1	0	6	(26)
Liu *et al*	1	1	0	1	1	1	1	0	6	(27)
Liu *et al*	1	1	0	1	1	1	1	0	6	(28)
Wang *et al*	1	1	0	1	1	1	1	0	6	(29)
Wang *et al*	1	1	0	1	1	1	1	0	6	(30)
Filipits *et al*	1	1	1	1	2	1	1	0	8	(31)
Wang *et al*	1	1	0	1	1	1	1	0	6	(32)
Zhang *et al*	1	0	0	1	1	1	1	0	5	(33)
Zuo *et al*	1	1	0	1	1	1	1	0	6	(34)
Sun *et al*	1	1	0	1	1	1	1	0	6	(35)
Zhang *et al*	1	1	0	1	1	1	1	0	6	(36)
Li *et al*	1	1	1	1	1	1	1	0	7	(37)
Li *et al*	1	1	0	1	1	1	1	1	7	(38)
Hao *et al*	1	1	0	1	1	1	1	1	6	(39)
Yoh *et al*	1	1	0	1	1	1	1	1	7	(40)
Guo *et al*	1	1	0	1	1	1	1	0	6	(41)
Li *et al*	1	1	0	1	1	1	1	0	6	(42)
Xia *et al*	1	0	0	1	1	1	1	0	5	(43)
Peng *et al*	1	0	0	1	1	1	1	1	6	(44)
Huo *et al*	1	1	0	1	1	1	1	0	6	(45)
Han *et al*	1	1	0	1	1	1	1	0	6	(46)
Yang *et al*	1	1	0	1	1	1	1	0	6	(47)
Rui *et al*	1	0	0	1	1	1	1	0	5	(48)
Wang *et al*	1	1	0	1	1	1	1	0	6	(49)
Wang *et al*	1	1	0	1	1	1	1	0	6	(50)
Xu *et al*	1	1	0	1	1	1	1	0	6	(51)
Peng *et al*	1	1	0	1	1	1	1	0	6	(51)
Zhan *et al*	1	1	0	1	1	1	1	0	6	(53)
Wright *et al*	1	1	1	1	1	1	1	0	7	(54)
Xu *et al*	1	1	0	1	1	1	1	0	6	(55)

[a]Total score ranges from 0 to 9 stars.

Table III. Analysis of the associations between expression of the multidrug resistance-associated protein 1 gene and the clinical parameters in patients with non-small cell lung cancer.

Parameter	n	Heterogeneity analysis		Effects model	OR (95% CI)	P-value
		I^2 value, %	P-value			
Age, years	4	3.7	0.374	Fixed	0.97 (0.72-1.30)	0.839
Sex	8	8.1	0.368	Fixed	0.99 (0.76-1.30)	0.970
Smoking status	3	0.0	0.658	Fixed	2.54 (1.17-5.54)	0.019
Histological origin	11	28.0	0.178	Fixed	5.54 (3.69-8.32)	0.000
Pathological type	27	59.2	0.000	Random	1.58 (1.16-2.17)	0.004
Cytological grade	16	56.2	0.003	Random	1.44 (0.99-2.08)	0.058
Lymph node metastasis	14	24.6	0.188	Fixed	1.32 (1.09-1.61)	0.005
Clinical stage	18	28.8	0.123	Fixed	1.36 (1.11-1.66)	0.003
Response to chemotherapy	5	0.0	0.664	Fixed	0.41 (0.23-0.72)	0.002
1-year survival rate	8	0.0	0.548	Fixed	0.76 (0.56-1.03)	0.073
3-year survival rate	8	62.5	0.009	Random	0.40 (0.23-0.68)	0.001

OR, odds ratio; I^2, variation in OR attribuTable to heterogeneity; CI, confidence interval.

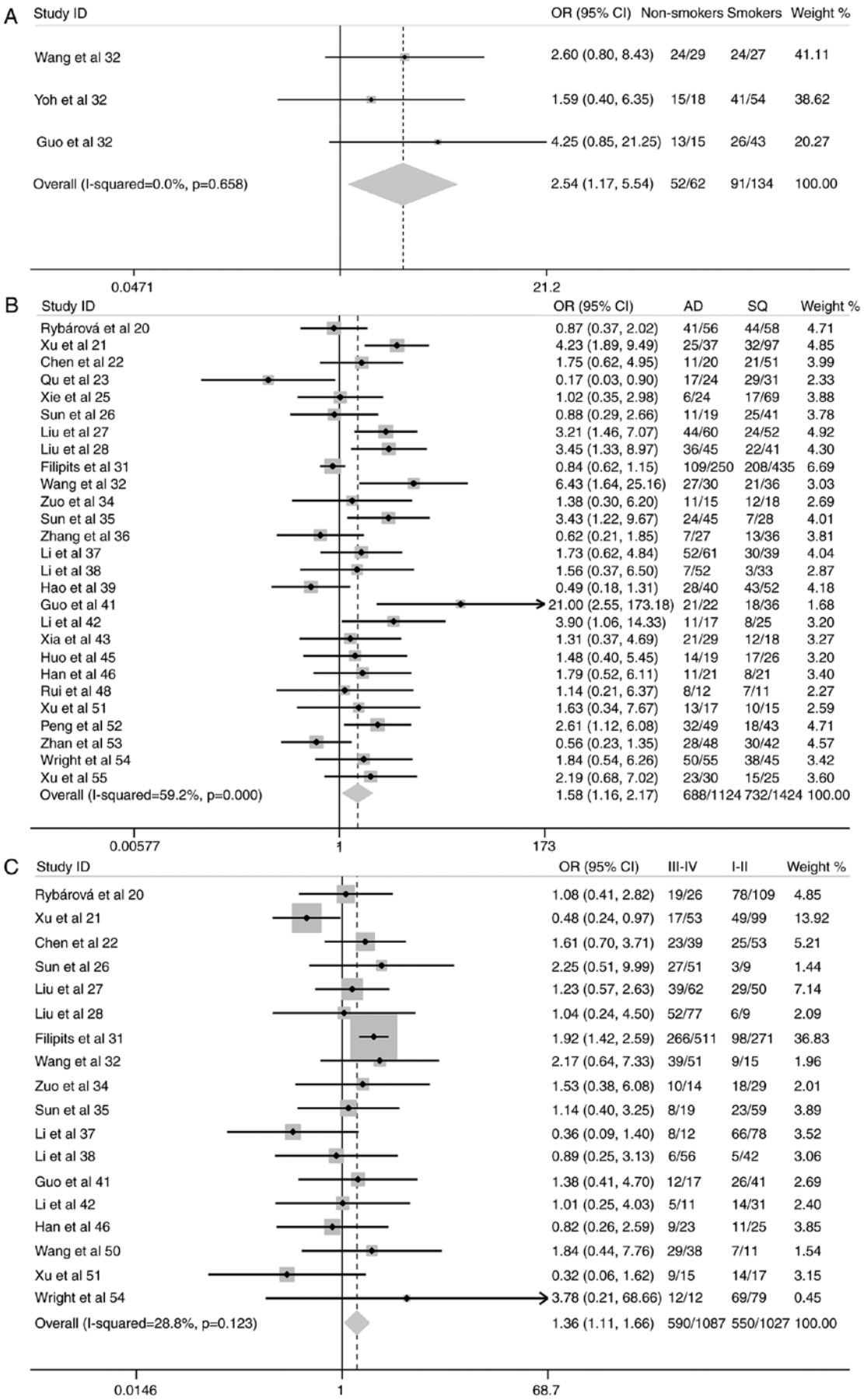

Figure 2. Forest plots of associations between MRP1 and smoking status, pathological type and clinical stage in patients with non-small cell lung cancer. (A) Association between MRP1 and smoking status. (B) Association between MRP1 and pathological type. (C) Association between MRP1 and clinical stage. MRP1, multidrug resistance-associated protein 1; AD, adenocarcinoma; SQ, squamous cell carcinoma; I-II, clinical stage I and II; III-IV, clinical stage III and IV; OR, odds ratio; CI, confidence interval.

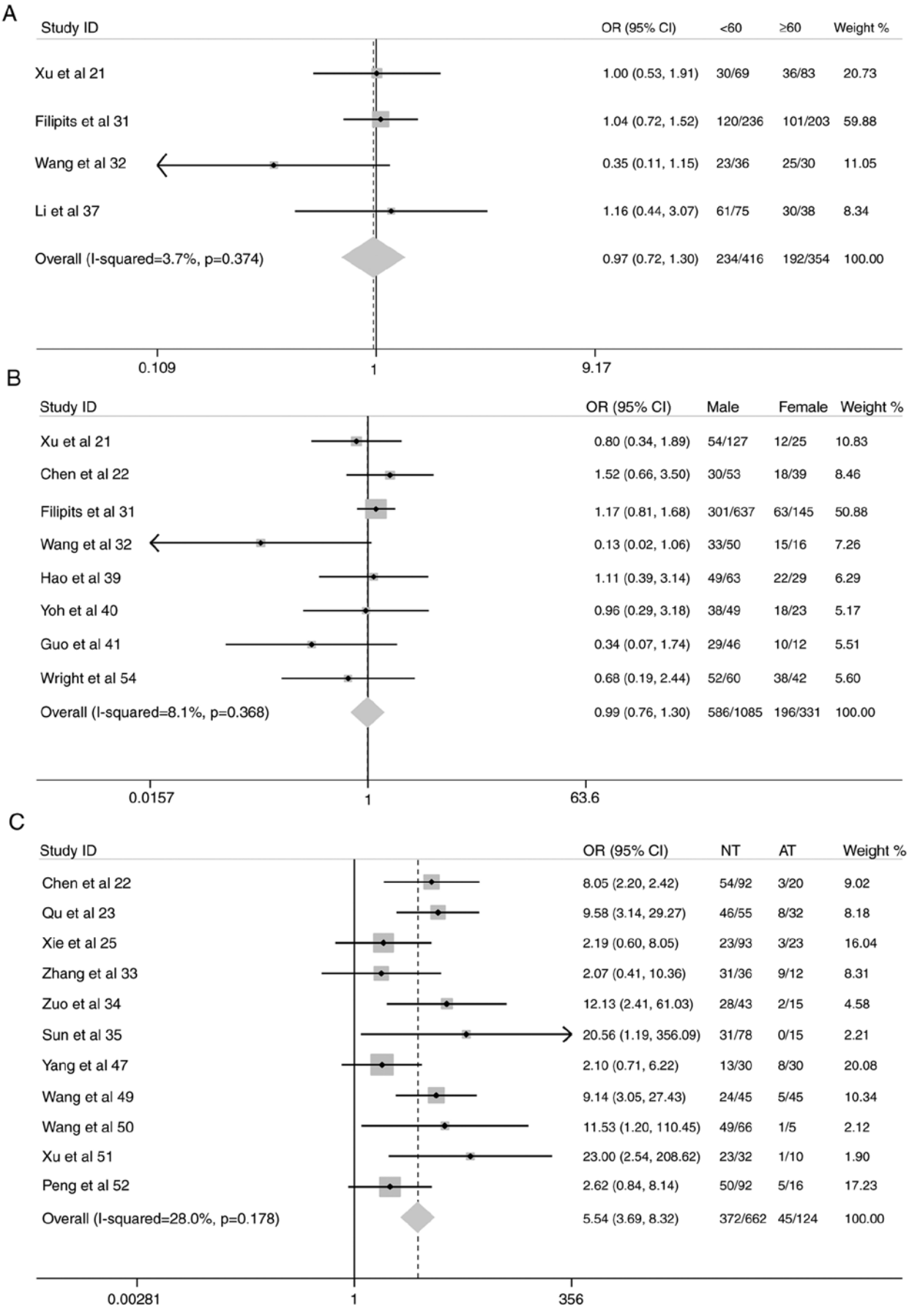

Figure 3. Forest plot of associations between MRP1 and age, sexand histological origin in patients with NSCLC. (A) Association between MRP1 and age. (B) Association between MRP1 and sex. (C) Association between MRP1 and histological origin. MRP1, multidrug resistance-associated protein 1; NSCLC, non-small cell lung cancer; NT, NSCLC tumor tissue; AT, adjacent non-cancerous tissue; OR, odds ratio; CI, confidence interval.

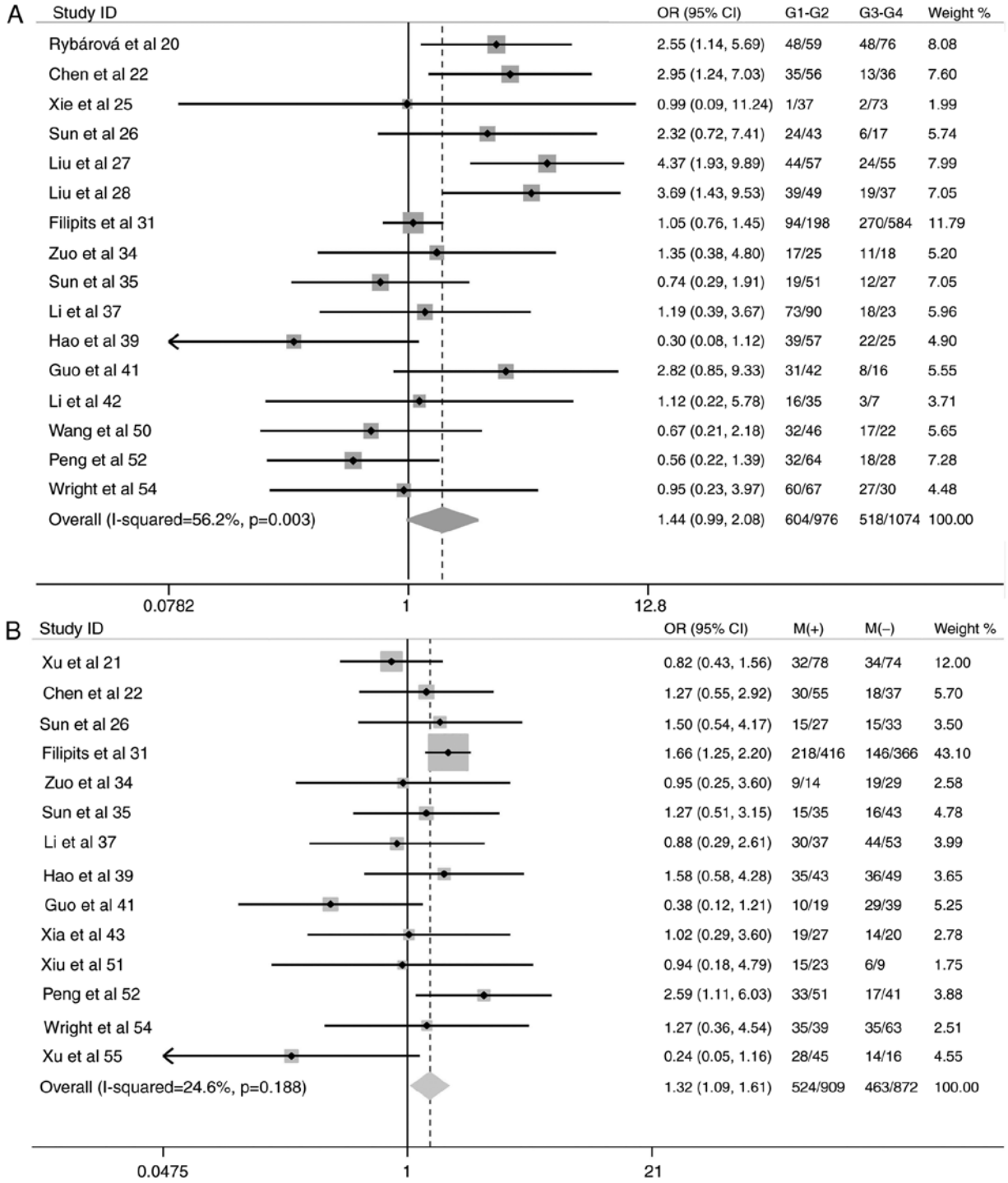

Figure 4. Forest plot of associations between MRP1 and cytological grade and lymph node metastasis in patients with non-small cell lung cancer. (A) Association between MRP1 and cytological grade. (B) Association between MRP1 and lymph node metastasis. MRP1, multidrug resistance-associated protein 1; G1-G2, grades 1 and 2; G3-G4, grades 3 and 4; M(+), positive for lymph node metastasis; M(-), negative for lymph node metastasis; OR, odds ratio; CI, confidence interval.

Discussion

The present meta-analysis of 36 clinical studies of 3,278 patients with NSCLC suggested that increased expression of the MRP1 gene in NSCLC patients is significantly associated with the following specific subgroups: Non-smokers, adenocarcinoma and advanced clinical stage. Increased MRP1 gene expression is also significantly associated with decreased 3-year survival rate (P=0.001) and a compromised response to chemotherapy (P=0.002). These findings highlight the updated clinical implications of MRP1 gene expression in patients with NSCLC. To the best our knowledge, this is by far the most extensive study summarizing current evidence on the clinicopathological relevance of MRP1 in NSCLC patients.

The present study showed that non-smoking patients with NSCLC tend to have increased expression of MRP1 compared with their smoking counterparts. Apart from the data extracted from the included studies (32,40,41), there is

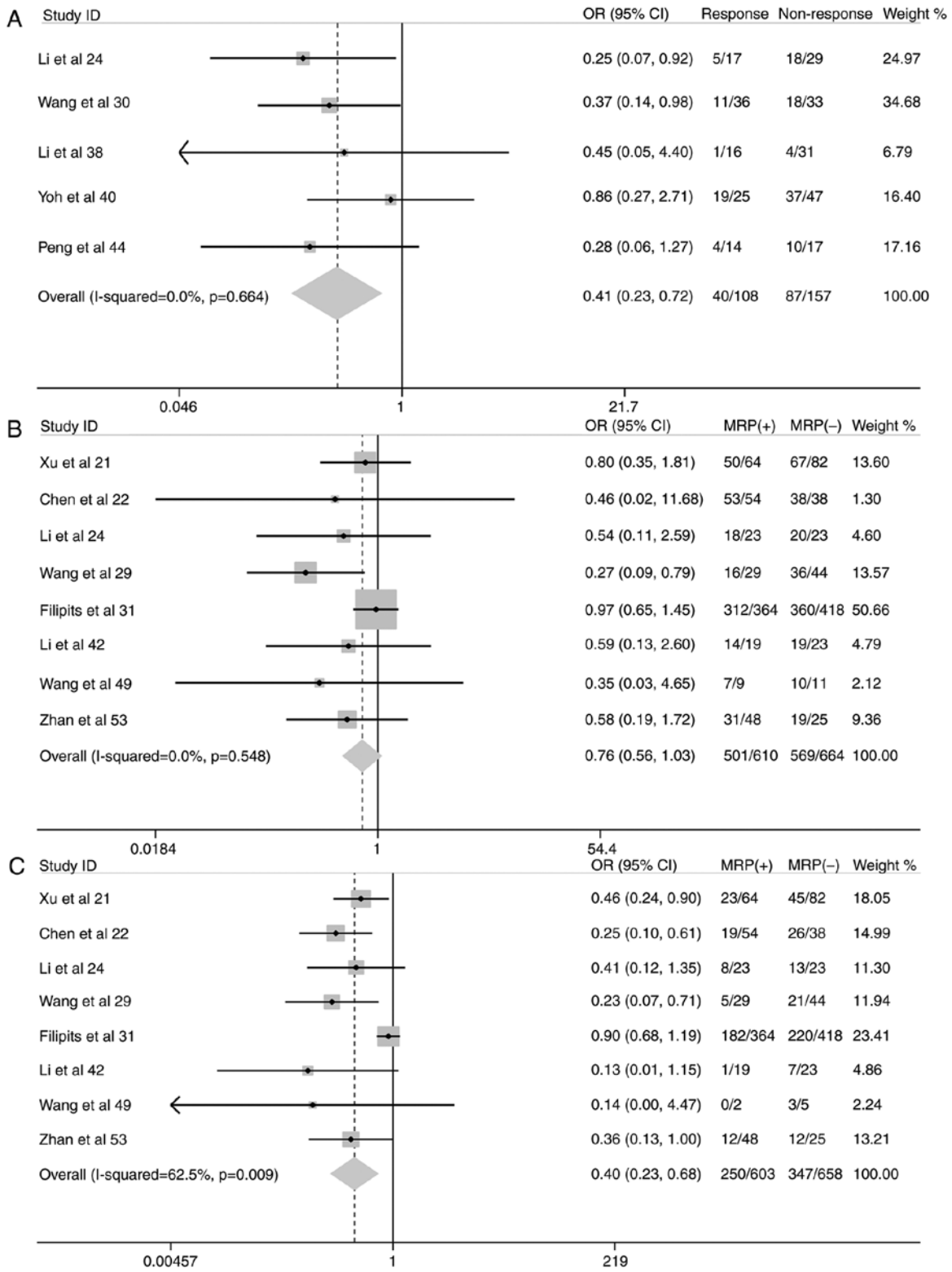

Figure 5. Forest plot of associations between MRP1 and response to chemotherapy, 1-year survival rate and 3-year survival rate in patients with non-small cell lung cancer. (A) Association between MRP1 and response to chemotherapy. (B) Association between MRP1 and 1-year survival rate. (C) Association between MRP1 and 3-year survival rate. MRP1, multidrug resistance-associated protein 1; MRP1(+), elevated expression of MRP1; MRP1(-), low expression of MRP1; OR, odds ratio; CI, confidence interval.

currently no other study directly comparing the MRP1 expression in smoking and non-smoking patients with NSCLC. An *in vitro* study has demonstrated that cigarette smoke extracts diminish MRP1 activity in the human bronchial epithelial cells (56). A previous study by Leslie *et al* (57) also suggested that MRP1-mediated activity of uptake of organic anions can be inhibited by nicotine glucuronide conjugates. By contrast, another study claimed that the physiological functions of MRP1 are unlikely to be substantially decreased by nicotine glucuronide metabolites at concentrations achievable

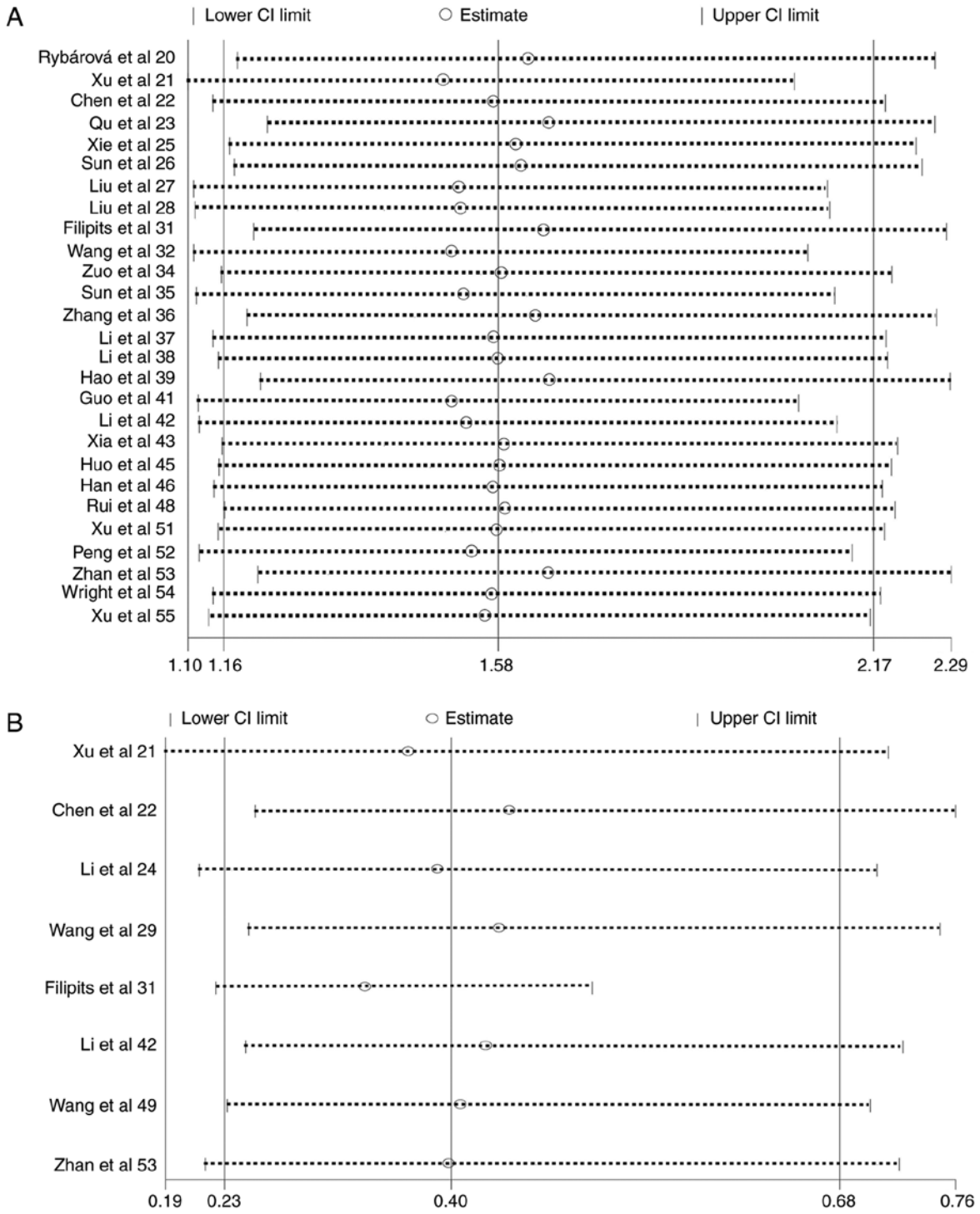

Figure 6. Sensitivity plot corresponding to the correlations between multidrug resistance-associated protein 1 and pathological type and three-year survival rate. (A) Pathological type sensitivity analysis. (B) The 3-year survival rate sensitivity analysis. CI, confidence interval.

in human serum (58). More convincing data derived from studies based on patients with chronic obstructive pulmonary disease (COPD), a condition strongly associated with smoking history, found that diminished MRP1 expression was observed in the bronchial epithelium and lung tissues in the COPD group (59,60). It was concluded that MRP1 appeared to be a protective protein for COPD development. The exact role of increased MRP1 expression in non-smoking NSCLC patients is unclear and requires further study.

In agreement with other previous studies, significantly enhanced MRP1 expression in lung cancer tissue of NSCLC patients was observed (OR, 5.54; 95% CI, 3.69-8.32; P<0.0001), in comparison to the non-tumor pulmonary tissue adjacent to the tumor, in the present study. The published studies have overwhelmingly shown that MRP1 is highly expressed and functionally active in NSCLC cells, and is potentially associated with negative treatment outcomes in NSCLC patients (12-14,22,24,36,49,52). It is unclear how MRP1

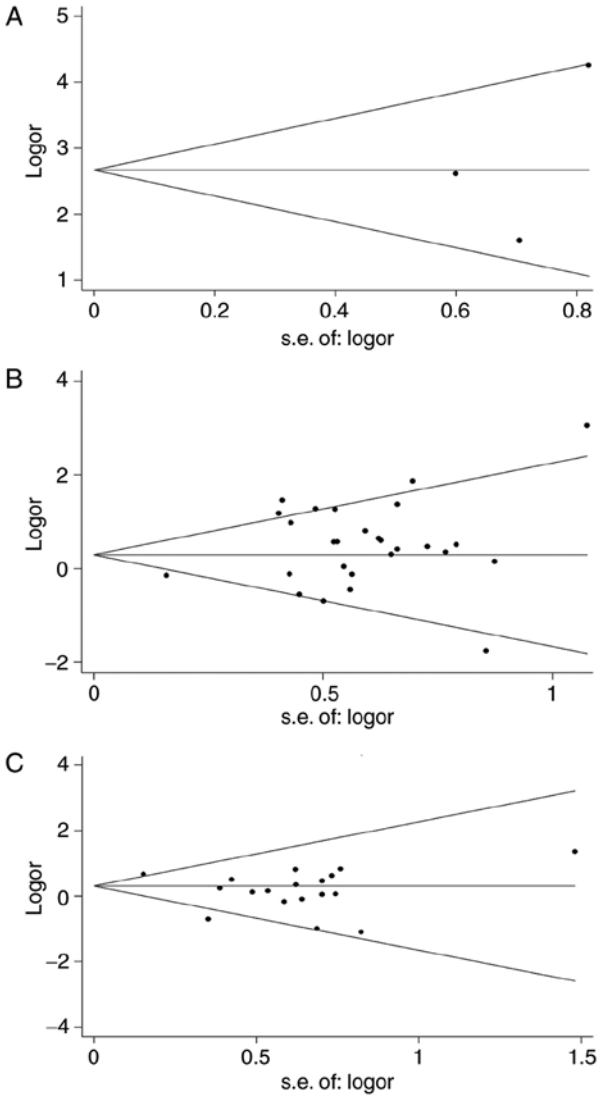

Figure 7. Begg's funnel plots of multidrug resistance-associated protein 1 for the assessment of potential publication bias. (A) Smoking status (smokers vs. non-smokers). (B) Pathological type (adenocarcinoma vs. squamous cell carcinoma). (C) Clinical stage (III-IV vs. I-II). OR, odds ratio.

expression is regulated in NSCLC (61) and in other tissues (12). However, increased expression of MRP1 in NSCLC tissues could be explained in two ways. Firstly, it maybe a result of p53 mutant expression in NSCLC, as mutant p53-positive NSCLC in patients has shown a significant correlation with MRP1 overexpression (61,62). Secondly, it is possible that MRP1 could be induced by cancer chemotherapy or radiotherapy, as evidence has clearly shown that the mRNA levels of MRP1 in recurrent tumors and residual tumors following chemotherapeutic treatment are higher than those in untreated primary tumors (63,64). These explanations can also be applied to other findings from the current study, which showed an increase in MRP1 gene expression was associated with patients in advanced clinical stages (stage III-IV) and with lymph node metastases. It should be noted that these findings contradict the results from one study by Berger *et al* (64), which stated that MRP1 expression levels were highest in stage I and declined with advanced stage. Although this could be associated with ethnicity, the discrepancy is mainly due to the differences in sample size. The present results were derived from 19 studies involving 2,114 patients, while the sample size in the study by Berger *et al* was limited to 126 patients. Therefore the results presented in this report are much more extensive and show better representation.

MRP1(+) patients with NSCLC were found to have a compromised response to platinum-based chemotherapy and decreased 3-year survival rates when compared with their MRP1(-) counterparts. A clear trend was observed linking MRP1 expression to decreased 1-year survival, although this was not statistically significant (P=0.07). It has been demonstrated that MRP1 is highly expressed in patients with NSCLC and associated with a defect in platinum accumulation in cisplatin-resistant cell lines (65,66). Triller *et al* (67) observed a significant negative correlation between MRP1 expression and a more favorable response rate to chemotherapy in patients with NSCLC. Similarly, it is worth noting that increased expression of ABCG4 and transforming growth factor β receptor type 2 are also identified as novel poor prognostic factors of chemotherapy in NSCLC patients (68,69). It is possible that an increase in MRP1-mediated efflux of anti-neoplastic agents reduces the intracellular concentration, and thereby decreases the therapeutic efficacy of the agents. RNA interference-based knockdown of MRP1 reversed MDR efficiently by decreasing the efflux ability and increasing the DDP-induced apoptosis in A549/DDP cells (70). These studies provided a solid ground for the clinical use of the MRP1 inhibitor for the treatment of NSCLC patients with enhanced expression of MRP1. It is predicted that the combined use of MRP1 inhibitor(s) with anti-neoplastic agents would increase the intracellular drug concentration, and thus possibly increase the curative effect of the agents. Indeed, a recent study demonstrated that meloxicam, a COX-2 inhibitor, increased the intracellular accumulation of doxorubicin and enhanced doxorubicin-induced cytotoxicity in the human lung cancer A549 cell line via downregulation of MRP1 (71).

The present analysis clearly shows that the MRP1 expression in patients with adenocarcinoma is significantly higher as opposed to that in patients with squamous cell carcinoma, and this is consistent with numerous other studies (72-77). However, it is noted that in a few studies, no significant differences in MRP1 expression between different histological subtypes of NSCLC could be detected (64). In the present study, 61.2% (688/1,124) of patients with adenocarcinoma were MRP1(+), whereas the presence of epidermal growth factor receptor (EGFR) mutations ranged from 40.3 to 64.5% in patients with adenocarcinoma (6,76,78-82). Currently, mutant EGFR is considered as a good predictor of clinical response to tyrosine kinase inhibitors (TKIs). Administration of TKIs has been shown to have a superior therapeutic value to chemotherapy regimens in non-smoking Asian patients with pulmonary adenocarcinoma harboring a higher rate of EGFR mutations (76,83). The use of TKIs is indicated in NSCLC patients harboring EGFR mutations, rather than in those with increased expression of the MRP1 gene. However, there is certain evidence suggesting that EGF induces MRP1 gene expression and increases MRP1 promoter activity (84). TKIs have become promising MRP1 inhibitors. Ibrutinib, a Bruton's TKI, was shown to significantly increase the efficacy of chemotherapeutic agents in MRP1-overexpressing cells of leukemia

by antagonizing the efflux function of the MRP1 transporter (9). In addition, interactions of human MRP1 with TKIs, including imatinib and AG1393, have also been reported to inhibit its transportation activity (9,85). Although the exact association between EGFR and MRP1 in NSCLC is uncertain at present, it is tempting to postulate that the combined use of TKIs and MRP1 inhibitors may have a synchronous effect in treating NSCLC patients with increased gene expression for MRP1.

One of the potential limitations of this meta-analysis is its potential risk of sampling bias: 34 out of the 36 studies were conducted in Asia, with the majority of the patients being Chinese. Therefore it is unknown whether a study with larger samples covering multiple ethnic groups would lead to the same conclusions. Another potential limitation is the relatively small sampling size in certain subgroups, including the MRP1 gene expression and smoking status subgroup, which contained 3 studies and only 196 patients (Fig. 2). Although the results of this meta-analysis are overall extremely promising, larger-scale clinical studies would provide more robust evidence-based results.

In conclusion, increased expression of the MRP1 gene is associated with a non-smoking status, adenocarcinoma, advanced clinical stages and a poor prognosis to chemotherapy in patients with NSCLC, indicating that the MRP1 gene serves a significant role in the development of NSCLC. The present results suggest further research on the implications of MRP1 expression in NSCLC is required to validate the important clinical significance of MRP1 expression that may influence the treatment of NSCLC. In particular, the fact that enhanced MRP1 expression strongly associates with a poor prognosis and advanced clinical stages of NSCLC provides a compelling foundation to continue investigating the potential use of MRP1 as a biomarker/clinical indicator for NSCLC.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

YL, MX, PH and PTYW conceived and designed the study. PH and PTYW executed the study. YL, MX, PH and PTYW analyzed and interpreted the data, and drafted and edited the manuscript. QZ, LH, WN and SC analyzed and interpreted data, and revised and edited the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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