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Original Article

Prognostic values of prevalent gene mutations in intrahepatic cholangiocarcinoma

Zheng Wang*, Xiang-Yu Wang*, Jian-Hua Li, Wen-Wei Zhu

*Department of Surgery, Huashan Hospital, Fudan University, Shanghai 200040, China. *Equal contributors.*

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Abstract: Intrahepatic cholangiocarcinoma (ICC) is a complicated and fatal cancer. With the development of sequencing technologies, *TP53, KRAS* and *IDH1/2* have been identified as frequently mutant genes in ICC. However, the prognostic value of these mutations remained uncertain. In order to clarify the prognostic values of these mutant genes in ICC, a systematic search of Pubmed, Embase and Web of Science was performed. Publications which met inclusion and exclusion criteria were finally included. The hazard ratio (HR) and 95% confidence interval (CIs) of *TP53*, *KRAS*, and *IDH1/2* mutation for overall survival (OS) was extracted from each eligible study. Also, the odds ratio (OR) and 95% CI of these gene mutations for clinical features was extracted. The I^2 and χ^2 tests were applied to assess heterogeneity between the enrolled studies, and $P<0.05$ indicated statistical significance. If $P>0.05$, we used a fixed-effects model to calculate the pooled HR to assess the association between gene mutation and overall survival; and if $P<0.05$, we used a random-effects model. In addition, the pooled OR was calculated to investigate the correlation between gene mutation and clinical features in ICC. A total of 16 studies composing of 1,516 ICC patients were included in the final analysis. Eight articles studied the relationship between *TP53* mutation and overall survival, indicating that patients with *TP53* mutation had poorer prognosis (HR=2.91, 95% CI: 1.67-5.09). And meta-analysis of eleven studies revealed that *KRAS* mutation was an adverse prognostic predictor in ICC (HR=2.30, 95% CI: 1.70-3.12). The result of seven studies showed that *IDH1/2* mutation had no impact on prognosis of ICC patients (HR=1.02, 95% CI: 0.62-1.67). However, the subgroup analysis suggested that *IDH1/2* mutation was a favorable prognostic factor in patients from Asia (HR=0.53, 95% CI: 0.33-0.87), but it could shortened overall survival in patients from America and Europe (HR=1.41, 95% CI: 1.05-1.90). We also found that *KRAS* mutation was associated with progressive tumor stage (III-IV) (OR=0.31, 95% CI: 0.15-0.63), and that patients with *IDH1/2* mutation tended to have poor tumor differentiation (OR=0.41, 95% CI: 0.19-0.90). These findings indicated there are different prognostic roles of *TP53*, *KRAS* and *IDH1/2* mutations in ICC.

Keywords: Intrahepatic cholangiocarcinoma, *TP53*, *KRAS*, *IDH*, mutation, prognosis

Introduction

Intrahepatic cholangiocarcinoma (ICC) is a highly malignant subtype of biliary cancers originating from the epithelium of the intrahepatic bile duct [1]. It is also the second most common primary hepatic malignancy following hepatocellular carcinoma (HCC) [2]. In recent years, the incidence and mortality of ICC are on the rise globally [3-5], with the highest prevalence in southeast and eastern Asia countries [6]. A wide variety of risk factors have been reported to be closely related to ICC carcinogenesis, such as HBV/HCV infection, hepatolithiasis, liver fluke infection and primary sclerosing cholangitis (PSC) [7, 8], indicating the complicated oncogenic mechanism of this solitary malignancy.

Clinically, surgical resection is the only curative treatment for early-stage ICC [9]. However, ICC patients usually have insidious onset and are mostly diagnosed at advanced stages. Despite advances in early detection and surgical treatment in these years, the prognosis of ICC after curative resection remains extremely dismal because of high recurrence rates and lacking of effective adjuvant therapies.

In this regards, a better understanding of the oncogenic mechanism underlying ICC tumorigenesis and progression is needed. As recent

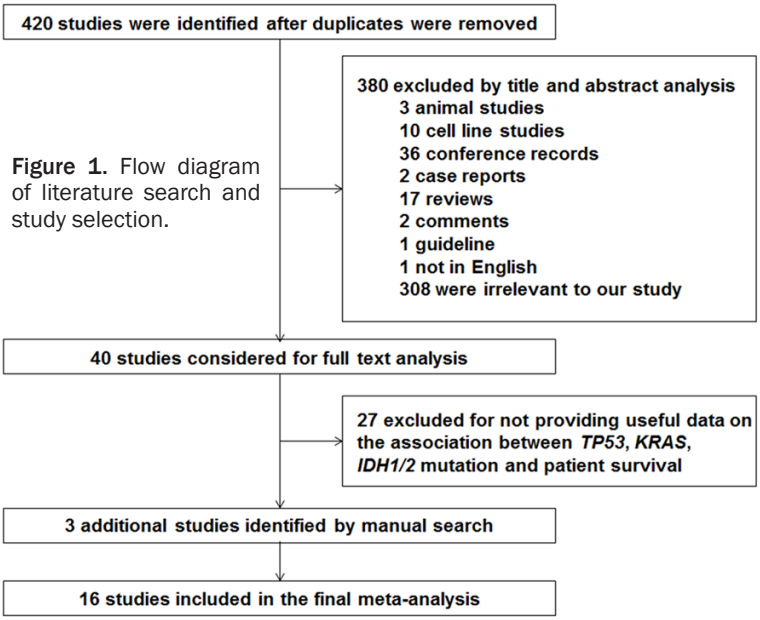

Figure 1. Flow diagram of literature search and study selection.

development of next generation sequencing, many genetic mutations of oncogenes and tumor suppressors were identified in ICC, among which the most prevalent mutant genes are *TP53*, *KRAS* and *IDH1/2* [10]. Many studies have evaluated whether these genetic lesions may serve as prognostic factors for survival in patients with ICC. However, the results of these studies are inconclusive and no consensus has been reached. It is unknown whether differences in these investigations have been mostly due to their limited sample size or heterogeneity. Thus, we conducted a meta-analysis of all available studies relating *TP53*, *KRAS*, *IDH1/2* mutation status with the clinical outcome in patients with ICC.

Methods

Literature search

The Pubmed, Embase and Web of Science were searched systematically for the relevant publications until February 1, 2016. The search terminologies were as follows: "cholangiocarcinoma", "liver cancer", "bile tract cancer", "bile duct cancer", "biliary cancer", "*TP53*", "*KRAS*", "*IDH*", "mutation", "survival", "prognosis". These terminologies were used in all possible combinations without language restriction. The reference articles were also scanned manually for additional available publications.

Inclusion and exclusion criteria

Inclusion criteria were: (1) diagnosis of ICC was made by pathology; (2) mutations of *TP53*, *KRAS*, or *IDH1/2* were detected; (3) comparison group was the population with wild-type gene; and (4) sufficient data on the association of *TP53*, *KRAS*, and *IDH1/2* mutation with patient survival and other clinical features.

Exclusion criteria were: (1) reviews, case reports, conference records, comments, guidelines, animal studies and cell line studies; (2) not written in English; (3) not able to provide sufficient data on impact of *TP53*, *KRAS*, or *IDH1/2* on patient survival; and (4) duplicated information of previous publications.

Quality assessment and data extraction

Two reviewers made the assessment of literature qualities on the basis of Newcastle-Ottawa scale (NOS) [11], independently. Only the publications with the score more than six were included in the final meta-analysis.

And two reviewers extracted the data from the included publications, independently. All articles were double-checked and disagreements were solved by group meetings. For each paper, the following information was obtained: (1) first author name, (2) year of publication, (3) name of journal, (4) study region, (5) patient number, (6) male to female ratio, (7) median age, (8) comparison group, (9) gene mutation rate, (10) mutation detection method, (11) the hazard ratio (HR) and 95% confidence interval (CI) of *TP53*, *KRAS*, and *IDH1/2* mutation for overall survival (OS). If the survival information was only available in Kaplan-Meier curves, HR was obtained by the method of Parmar *et al* [12].

Statistical analysis

We used Stata version 12.0 to perform the meta-analysis of the enrolled studies. Pooled HR was calculated by synthesizing the HR and

Table 1. Characteristics of the studies included in the meta-analysis

First author	Year	Journal	Region	Patients	Male/Female	Median age	Comparison	Gene mutation (Rate)	Detection method	Outcome measures	Quality score
Anderson JB	2012	*Gastroenterology*	USA	104	48/56	64.0	Wildtype	*KRAS* (24.6%)	SGA	OS	8
Churi CR	2014	*Plos One*	USA	55	16/39	60.0 (24-84)	Wildtype	*TP53* (29.1%), *KRAS* (23.6%), *IDH1/2* (23.6%)	TGS	OS, RFS	9
Goyal L	2015	*Oncologist*	USA	104	47/57	61.0 (23-83)	Wildtype	*TP53* (2.9%), *KRAS* (8.7%), *IDH1/2* (28.8%)	SGA	OS	8
Jang S	2014	*Mod Pathol*	Korea	81	57/24	59.0 (33-78)	Wildtype	*TP53* (1.2%), *KRAS* (13.6%)	SS	OS	8
Jiao Y	2013	*Nat Genet*	USA	32	13/19	64.0 (35-84)	Wildtype	*TP53* (6%), *IDH1/2* (18.8%)	WES	OS	9
Nakamura H	2015	*Nat Genet*	Japan	137	89/48	NR	Wildtype	*TP53* (23.4%), *KRAS* (23.4%), *IDH1/2* (8.0%)	WES	OS	9
Ong CK	2012	*Nat Genet*	Singapore	54	34/20	55.0 (37-73)	Wildtype	*TP53* (44.4%), *KRAS* (16.7%)	WES	OS	9
Robertson S	2013	*Hum Pathol*	USA	54	17/37	64.0 (37-81)	Wildtype	*KRAS* (7.4%)	PS	OS	8
Ruzzenente A	2015	*Ann Surg Oncol*	Italy	35	20/15	66.6 (43-85)	Wildtype	*TP53* (5.7%), *KRAS* (8.6%), *IDH1/2* (20.0%)	TGS	OS	9
Simbolo M	2014	*Oncotarget*	Italy	70	42/28	64.8±11.6	Wildtype	*TP53* (8.6%), *KRAS* (15.7%), *IDH1/2* (20.0%)	SS	OS	9
Tannapfel A	2000	*Gut*	Germany	41	NR	NR	Wildtype	*KRAS* (54.0%)	SS	OS	8
Tannapfel A	2002	*J Pathol*	Germany	51	NR	NR	Wildtype	*TP53* (37.0%), *KRAS* (51.0%),	SS	OS	8
Tannapfel A	2003	*Gut*	Germany	69	NR	NR	Wildtype	*KRAS* (45.0%)	SS	OS	8
Wang P	2013	*Oncogene*	China	326	NR	NR	Wildtype	*IDH1/2* (10.4%)	SS	OS	9
Zhu AX	2014	*Ann Surg Oncol*	USA	200	111/89	63.0 (53-70)	Wildtype	*TP53* (2.5%), *KRAS* (8.6%), *IDH1/2* (20.0%)	SGA	OS	9
Zou S	2014	*Nat Commun*	China	103	65/38	54.5 (35-85)	Wildtype	*TP53* (38.2%), *KRAS* (16.7%), *IDH1/2* (4.9%)	WES	OS	9

NR: Not Reported; SGA: SNaPshot Genotyping Assay; TGS: Targeted Region Sequencing; SS: Sanger Sequencing; WES: Whole Exome Sequencing; PS: Pyrosequencing; OS: Overall Survival; RFS: Recurrence-Free Survival.

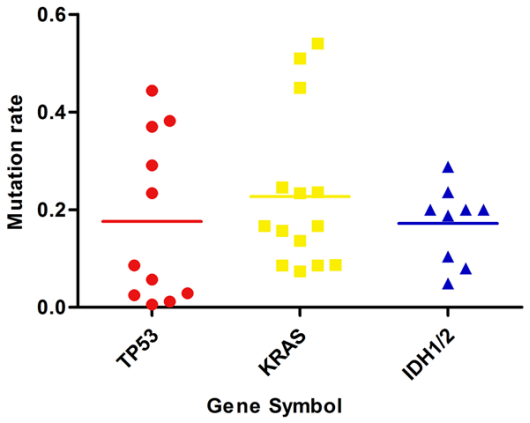

Figure 2. Mutation rate of most prevalent mutant genes.

95% CI from each article. The I^2 and χ^2 tests were applied to assess heterogeneity between the enrolled studies, and P<0.05 indicated statistical significance. If P>0.05, we used a fixed-effects model to calculate the pooled HR; and if P<0.05, we used a random-effects model. Begg's test and Egger's test were both performed to evaluate potential publication bias.

Results

Characteristics of the included studies

The flow diagram of literature search and study selection is shown in **Figure 1**. In the initial search, 420 studies were identified after duplicates were removed. On the basis of title and abstract, 380 articles (including 3 animal studies, 10 cell line studies, 36 conference records, 2 case reports, 17 reviews, 2 comments, 1 guideline and 308 irrelevant publications) were excluded. Among the 40 papers for full-text analysis, 27 articles failed to provide useful data and were excluded. In addition, three studies were identified by manual search. Ultimately, 16 studies were included in our meta-analysis.

As shown in **Table 1**, 16 studies composing of 1,516 ICC patients were included. The articles were published from 2000 to 2015. Eleven publications were studied in the region of America and Europe, and five researches were done in Asia. The sequencing methods varied among different papers, including Sanger Sequencing, SNaPshot Genotyping Assay, Pyrosequencing, Targeted Region Sequencing and Whole Exome Sequencing. All the studies were assessed according to the NOS scale, and were of high quality (score ≥6).

Impact of TP53 mutation on overall survival

The mutation rate of *TP53* ranged from 1.25% to 44.4%, and the average rate was 17.6% (**Figure 2**). Eight articles studied the association between *TP53* mutation and overall survival in ICC patients. The pooled HR was 2.91 (95% CI: 1.67-5.09, z=3.75, P<0.001) with heterogeneity (I^2=80.2%, P<0.001) (**Figure 3A**). This result indicated that patients with *TP53* mutation had poorer prognosis.

We also carried out subgroup analysis to assess the potential reason of heterogeneity. When stratified by study region, *TP53* mutation was an adverse prognostic factor for ICC in America and Europe (HR=3.22, 95% CI: 1.88-5.54) and no heterogeneity existed between studies (I^2=46.0%, P=0.016) (**Figure 3B**). But in Asian countries, *TP53* mutation had no impact on patient survival (HR=2.43, 95% CI: 0.78-7.59). When stratified by sequencing method, the pooled HR was 2.92 (95% CI: 1.33-6.41) in the group of next-generation sequencing and 2.90 (95% CI: 1.67-5.09) in the group of Sanger sequencing, respectively (**Figure 3C**). This suggested that *TP53* mutation was correlated with poorer survival regardless of sequencing method. All these results showed that *TP53* mutation indicated poorer prognosis in ICC, especially in patients from America and Europe.

Impact of KRAS mutation on overall survival

Fourteen studies reported the mutation rate of *KRAS* gene (ranged from 7.4% to 54.0%) with mean of 22.7% (**Figure 2**). Eleven publications analyzed the correlation between *KRAS* mutation and patient survival. The pooled HR was 2.30 (95% CI: 1.70-3.12, z=5.39, P<0.001) with heterogeneity (I^2=56.9%, P=0.010) (**Figure 4A**), which indicated that *KRAS* mutation was associated with shorter overall survival in ICC.

The subgroup analysis showed that *KRAS* mutation was an adverse factor for prognosis in both Asia (HR=2.67, 95% CI: 1.98-3.60) and America and Europe (HR=2.03, 95% CI: 1.27-3.26) (**Figure 4B**). In addition, *KRAS* mutation was related to poorer prognosis in the group of next-generation sequencing (HR=2.75, 95% CI: 2.08-3.64), but not in the group of Sanger

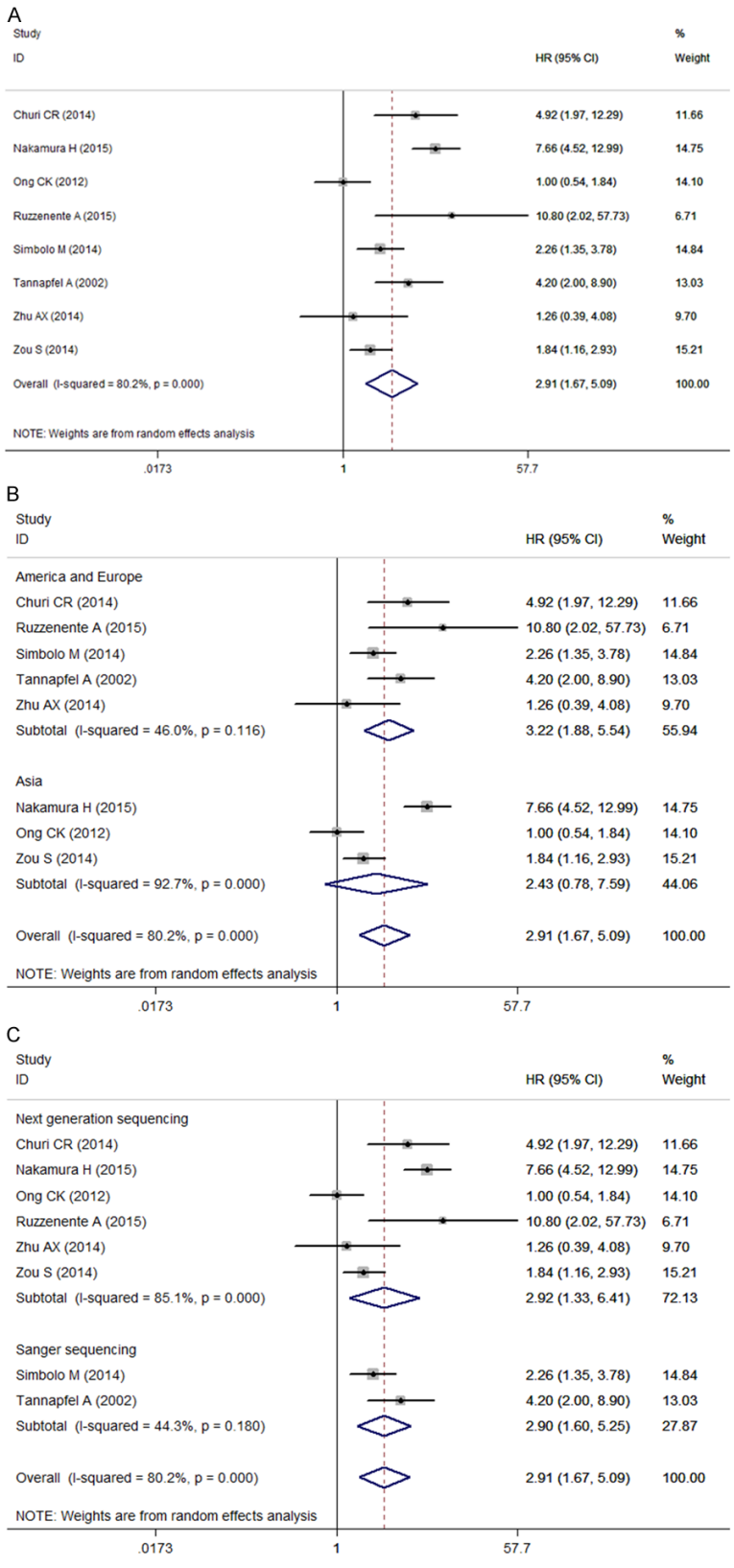

Figure 3. Forest plots of hazard ratios for the relationship between *TP53* mutation and overall survival. A: *TP53* mutation and overall survival; B: Subgroup analysis by study region; C: Subgroup analysis by sequencing method.

sequencing (HR=1.71, 95% CI: 0.93-3.12) (**Figure 4C**). These data suggested that patients with KRAS mutation had poorer prognosis than wild-type, regardless of region and nation.

Impact of IDH1/2 mutation on overall survival

IDH1/2 mutation was reported in nine publications, and the mean mutation rate was 17.2% (ranged from 4.9% to 23.6%) (**Figure 2**). And in total, seven studies focused on the relationship between *IDH1/2* mutation and overall survival in ICC. The meta-analysis suggested that *IDH1/2* mutation had no impact on prognosis of ICC patients (HR=1.02, 95% CI: 0.62-1.67, z=0.06, P=0.950) (**Figure 5A**). However, heterogeneity existed between the included studies (I^2=65.8%, P=0.007).

And we performed subgroup analysis and stratified the cases into two groups: America and Europe, and Asia. Interestingly, we found that *IDH1/2* mutation could shortened overall survival in patients from America and Europe (HR=1.41, 95% CI: 1.05-1.90) without heterogeneity (I^2=48.6%, P=0.120) (**Figure 5B**). However, *IDH1/2* mutation was a favorable predictive factor for survival in ICC patients from Asia (HR= 0.53, 95% CI: 0.33-0.87) and no heterogeneity existed (I^2= 0%, P=0.783). These findings indicated that the prognostic role of *IDH1/2* mutation varied among countries.

Correlation between gene mutation and clinical features

Association between gene mutations and clinicopatho-

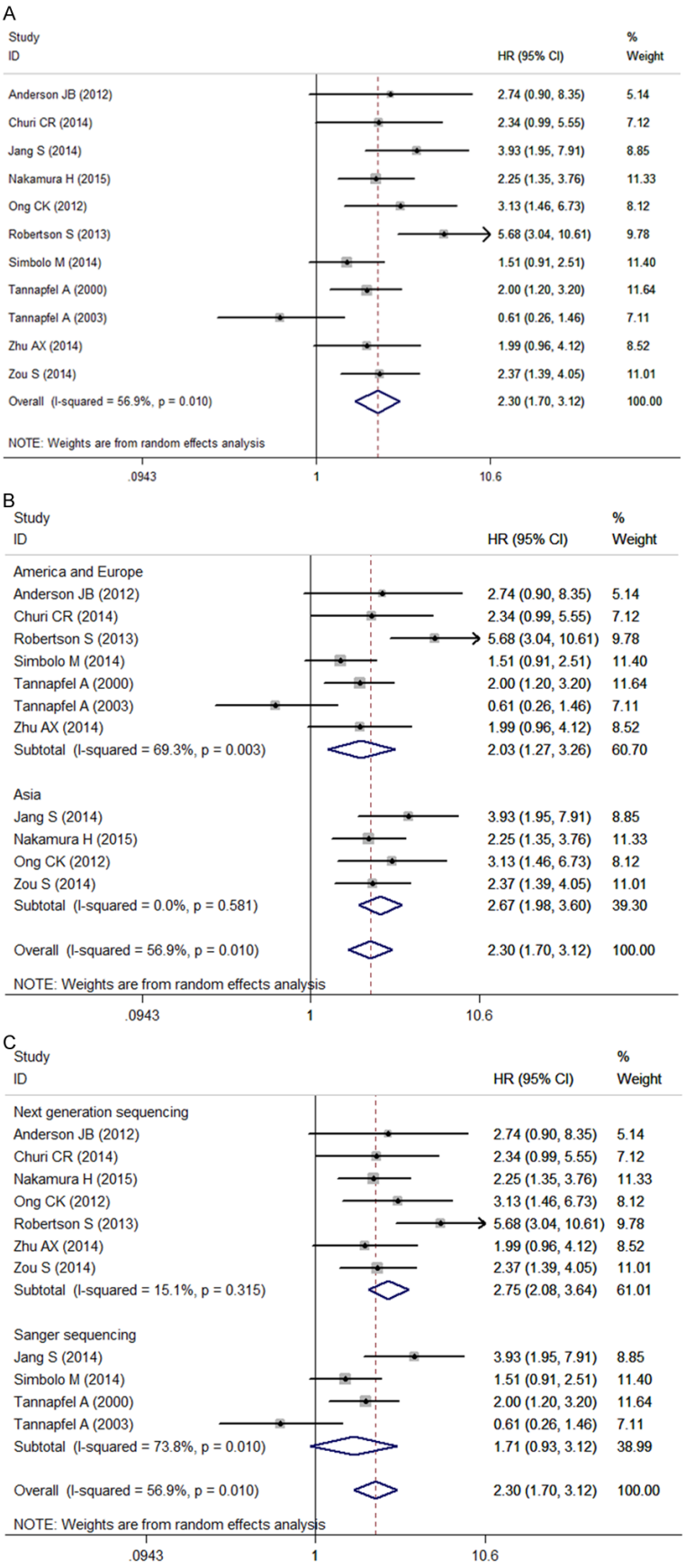

Figure 4. Forest plots of hazard ratios for the relationship between *KRAS* mutation and overall survival. A: *KRAS* mutation and overall survival; B: Subgroup analysis by study region; C: Subgroup analysis by sequencing method.

logical features was assessed in our meta-analysis, as well. The pooled odds ratio (OR) between *KRAS* mutation and tumor stage was 0.31 (95% CI: 0.15-0.63) without heterogeneity (I^2=0%, P=0.483) (**Figure 6A**). This result showed that *KRAS* mutation was associated with later tumor stage (III-IV). Moreover, patients with *IDH1/2* mutation tended to have poor tumor differentiation. The pooled OR was 0.41 (95% CI: 0.19-0.90) without heterogeneity (I^2=0%, P=0.740) (**Figure 6B**).

No significant correlation was found between *TP53*, *KRAS*, *IDH1/2* mutation and other clinical features in our research (**Table 2**).

Publication bias

We used Begg's test and Egger's test to assess potential publication bias. No publication bias was found for the meta-analysis of relationship between patient survival and mutation of *TP53* (Begg's test, P=0.386; Egger's test, P=0.657), *KRAS* (Begg's test, P=0.640; Egger's test, P=0.942) or *IDH1/2* (Begg's test, P=1.000; Egger's test, P=0.813) (**Figure 7**).

Discussion

In spite of the advancement in surgical technologies, ICC remains a complicated and intractable cancer. With the

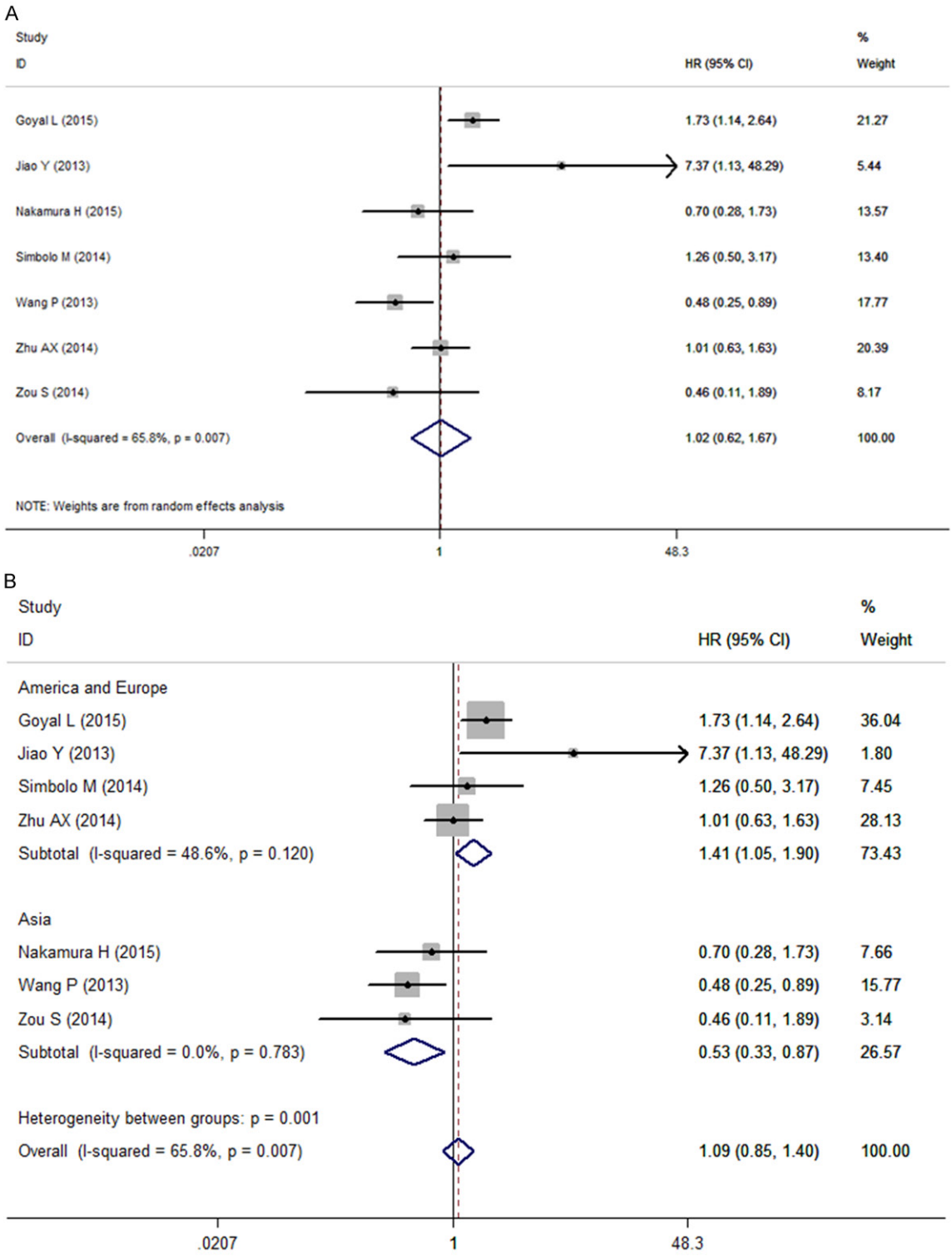

Figure 5. Forest plots of hazard ratios for the relationship between *IDH1/2* mutation and overall survival. A: *IDH1/2* mutation and overall survival; B: Subgroup analysis by study region.

development of sequencing methods, a series of mutant driver genes and key signaling pathways have been identified [13-15]. Among these genes, *TP53*, *KRAS* and *IDH1/2* are

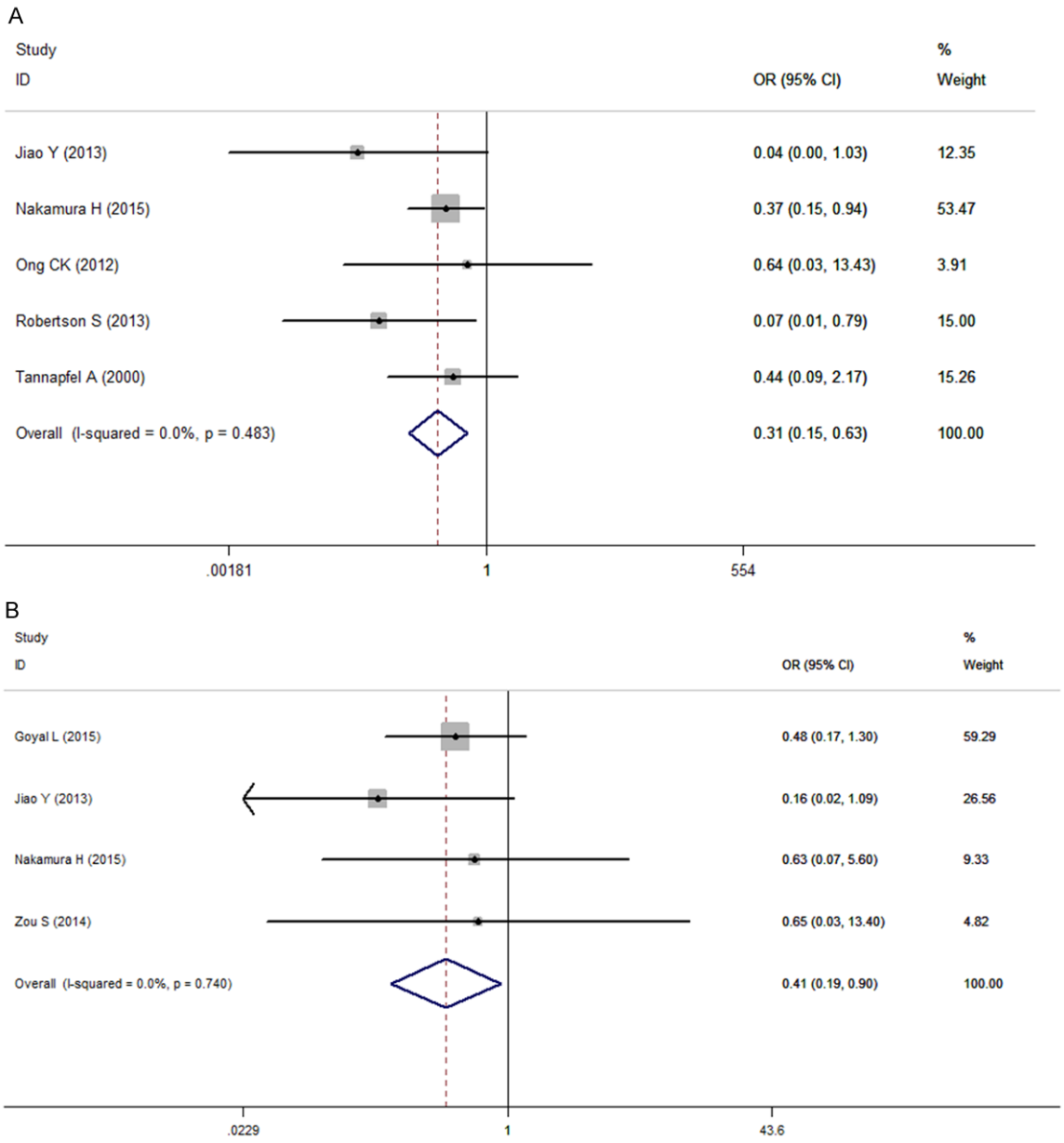

Figure 6. Forest plots of odds ratios for the relationship between gene mutation and clinical features. A: KRAS mutation and tumor stage; B: IDH mutation and tumor differentiation.

reported as the most prevalent mutant ones and significantly related to clinical outcome in ICC patients. Thus, we performed a meta-analysis to investigate the prognostic values of these gene mutations in ICC.

TP53 mutation, one of the most significant drivers in carcinogenesis and metastasis, was frequently reported in the gastrointestinal cancers [16, 17]. It was highly related to chromosome instability [18], and could interact with certain inflammation pathways [19]. Recent studies also uncovered the novel effects of *TP53* mutation on cancer metabolism and deregulated cancer epigenetic modifications. Consequently, *TP53* mutation was an adverse prognostic predictor in different cancer types [20, 21]. And in our research, we found that ICC patients with *TP53* mutation also had poorer prognosis.

KRAS mutation, about 20 years ago, was firstly identified as an important driver in ICC tumori-

Table 2. Meta-analysis of *TP53*, *KRAS*, *IDH1/2* mutation and clinicopathological features

Gene	Clinicopathological features	N	Cases	Analytical model	OR	95% CI	Heterogeneity	
							I^2 (%)	*P* Value
TP53								
	Gender (male vs. female)	3	223	FEM	1.36	0.70-2.62	0	0.761
	Differentiation (well/moderate vs. poor)	2	161	FEM	0.33	0.09-1.17	0	0.396
	Lymph node metastasis (yes vs. no)	2	185	FEM	1.40	0.73-2.69	0	0.978
	Staging (I-II vs. III-IV)	3	222	FEM	0.49	0.23-1.08	0	0.671
KRAS								
	Gender (male vs. female)	4	277	FEM	0.71	0.37-1.37	0	0.963
	HBV infection (yes vs. no)	2	189	FEM	1.45	0.33-6.40	0	0.976
	Differentiation (well/moderate vs. poor)	4	256	FEM	2.01	0.71-5.75	0	0.615
	Lymph node metastasis (yes vs. no)	4	280	FEM	1.42	0.75-2.67	0	0.393
	Staging (I-II vs. III-IV)	5	317	FEM	0.31	0.15-0.63	0	0.483
IDH1/2								
	Gender (male vs. female)	4	375	FEM	0.66	0.36-1.23	0	0.488
	HBV infection (yes vs. no)	2	237	FEM	0.37	0.07-1.93	67.2	0.081
	Differentiation (well/moderate vs. poor)	4	339	FEM	0.41	0.19-0.90	0	0.740
	Lymph node metastasis (yes vs. no)	4	370	FEM	0.80	0.42-1.53	50.5	0.109
	Vascular invasion (yes vs. no)	2	134	FEM	1.03	0.24-4.32	0	0.884
	Staging (I-II vs. III-IV)	3	270	REM	0.31	0.05-1.93	69.1	0.039

FEM: fixed-effects model; REM: random-effects model; OR: odds ratio; CI: confidence interval.

genesis [22]. In other gastrointestinal cancers such as colorectal carcinoma, *KRAS* mutation act as an adverse prognostic predictor [23], and lead to anti-response to monoclonal antibody therapy [24]. These reports highly supported our finding that ICC patients with *KRAS* mutation tended to have a poorer prognosis. And we also noticed that *KRAS* mutation was correlated with advanced tumor stage, which could partly explain for the reason why *KRAS* mutation could be an adverse factor for overall survival. In the research by Andersen *et al*., *KRAS* mutation, along with other gene signatures, could classify ICC patients into a poor prognosis subgroup [25]. Therefore, *KRAS* mutational status could be a useful tool to predict patient prognosis, make subtyping classifications, and benefit targeted therapies.

In recent years, recurrent *IDH1/2* mutations were identified in glioma [26, 27], acute myeloid leukemia (AML) [28], chondrosarcoma [29] and ICC [30, 31]. The *IDH1* and *IDH2* gene mutations predominantly occur in the hotspots of codon 132 and codon 172, respectively. Physiologically, IDH enzymes convert isocitrate to α-ketoglutarate (αKG). However, cancer-associated *IDH* mutations result in enzymes that convert αKG to 2-hydroxyglutarate (2 HG), which can competitively inhibit the activity of multiple αKG-dependent DNA and histone demethylases [32, 33]. These epigenetic alterations lead to changes in gene expression and impaired progenitor cell differentiation, which eventually result in tumorigenesis [33-35]. Consistently, our analysis revealed that the *IDH1/2* mutation status is significantly correlated with poor tumor differentiation in ICC. Clinically, *IDH1/2* mutation status defines a distinct subgroup of glioma with CpG island methylation phenotype (G-CIMP) and significantly better outcomes [32, 36]. According to our analysis, *IDH1/2* mutant ICCs indeed showed relative better prognosis in ICC patients from eastern Asian countries (China and Japan). However, studies from the western countries (America and Europe) demonstrated that *IDH1/2* mutation tend to shorten OS in ICC patients. This discrepancy could be partly explained by the great etiological difference between these areas, as hepatitis virus and liver fluke infection are the main etiological factors in eastern Asian countries [37, 38], while other factors such as PSC and nonalcoholic fatty liver disease are more prevalent in Western countries [39, 40].

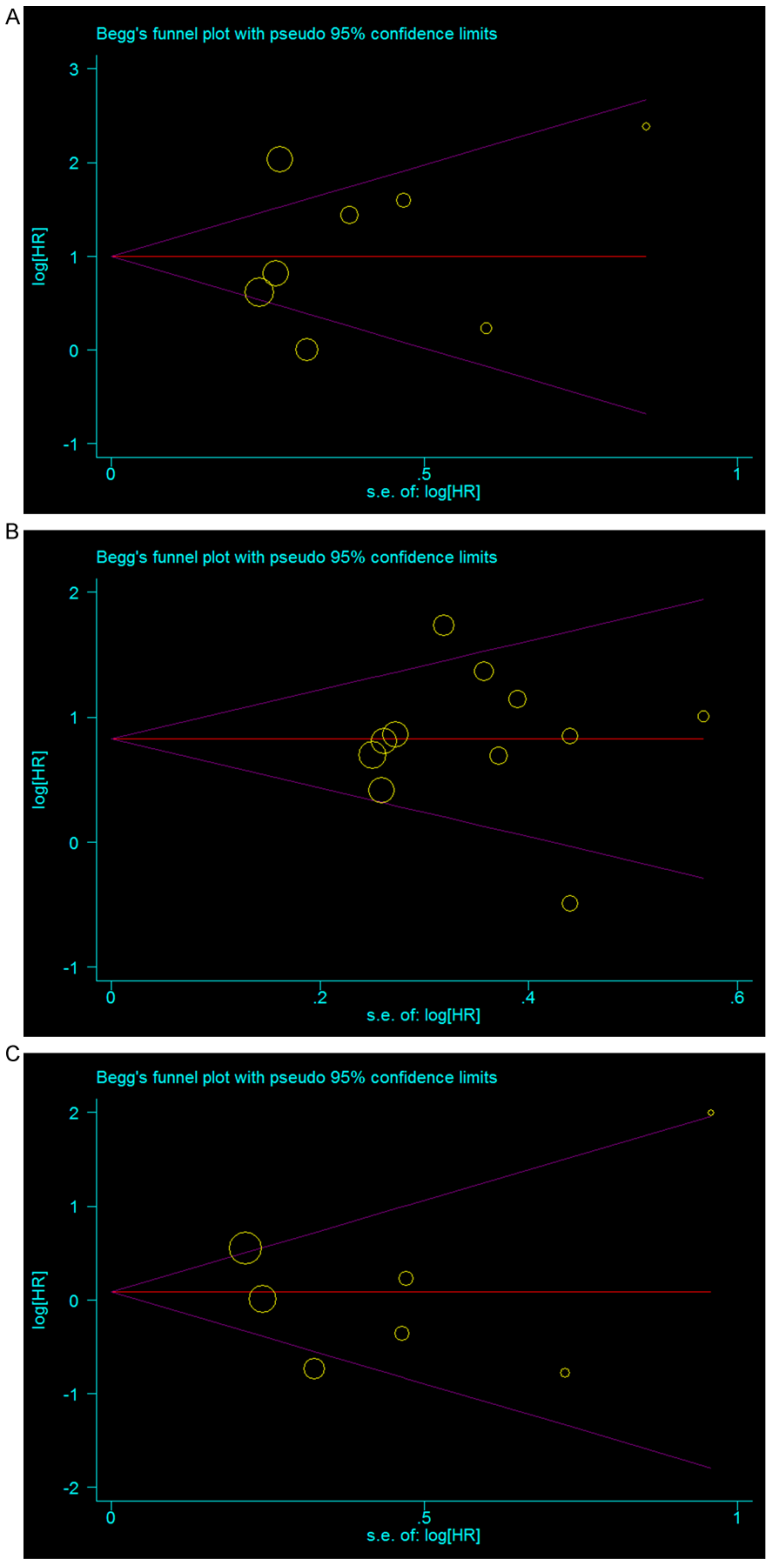

Figure 7. Funnel plots to assess the publication bias. A: *TP53* mutation and overall survival; B: *KRAS* mutation and overall survival; C: *IDH1/2* mutation and overall survival.

To ensure the quality of the enrolled studies, NOS scale was used to make the assessment and only high-quality publications (score ≥6) were finally included in our research. And, all the enrolled patients were diagnosed by the golden standard (pathology) and had a follow-up of at least three years. Since study regions included Asia (China, Japan, Korea and Singapore), Europe (Italy and Germany) and America, the enrolled cases were representative of ICC patients. Consequently, our results could provide authentic and solid evidence for the preoperation prediction and postoperation monitoring of intrahepatic cholangiocarcinoma.

Although our research was comprehensive and systematic, potential bias may exist. In the process of literature search, we only enrolled English publications. Potential language bias may exist although Begg's test and Egger's test showed no significant publication bias. Furthermore, only cohort studies were found in our meta-analysis and it was difficult to control the confounding bias in these researches.

In summary, our research suggested the different prognostic roles of *TP53*, *KRAS* and *IDH1/2* mutations in ICC, which provides optimized prognostic markers and further allows rational choices of molecular classification for this refractory malignancy.

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Disclosure of conflict of interest

None.

Address correspondence to: Wen-Wei Zhu, Department of Surgery, Huashan Hospital, Fudan Univer-

sity, 12 Middle Wulumuqi Road, Shanghai 200040, China. E-mail: westoolife@163.com

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