

ORIGINAL RESEARCH ARTICLE

Meta-analysis of the prognostic value of *KRAS* and *TP53* mutations in cholangiocarcinoma

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Abstract

Introduction: *KRAS* and *TP53* mutations are among the most common genetic mutations in cholangiocarcinoma.

Objective: This study aimed to explore the potential impact of these mutations on the survival prognosis of cholangiocarcinoma patients through a meta-analysis.

Methods: A systematic search was conducted in PubMed, Web of Science, Cochrane Library, Embase, CNKI, and Wanfang databases for cohort studies published up to April 2025 that investigated the relationship between *KRAS* and *TP53* mutations and patient prognosis. Two researchers independently performed the literature screening and data extraction. Meta-analysis was conducted using RevMan 5.4 and Stata 17.0, with assessment of publication bias.

Results: Twelve studies were included, involving a total of 1,126 patients, with 167 cases of *KRAS* mutations and 176 cases of *TP53* mutations. The results showed that both *KRAS* and *TP53* mutations were significantly associated with poorer survival prognosis in cholangiocarcinoma patients. For *KRAS* (hazard ratio (HR)=7.26; 95% confidence interval (CI): 6.10 – 9.81; $p < 0.05$) and *TP53* (HR=18.13; 95% CI: 11.24 – 25.32; $p < 0.05$), the presence of mutations predicted an adverse prognosis.

Conclusion: While these findings suggest that *KRAS* and *TP53* mutations are associated with worse survival in cholangiocarcinoma, the limited number and quality of the included studies warrant further high-quality research to validate these associations.

Keywords: Cholangiocarcinoma; *KRAS* mutation; *TP53* mutation; Prognosis; Meta-analysis; Cohort studies

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1. Introduction

Cholangiocarcinoma, a malignant tumor of the bile duct, can be anatomically classified into intrahepatic and extrahepatic types. It currently accounts for approximately 3% of all gastrointestinal cancers.¹ In recent years, the incidence and mortality rates of cholangiocarcinoma have been rising globally, particularly in Asia.² The primary treatment for early-stage cholangiocarcinoma is surgical resection; however, due to non-specific symptoms and subtle clinical manifestations in the early stages, 50 – 60% of patients are diagnosed at an advanced stage, with surgery suitable for only 15% of these cases.³ Current research shows that the 5-year survival rate for advanced cholangiocarcinoma is extremely low, making early diagnosis and prognostic assessment critical for clinical decision-making.⁴

KRAS and *TP53* mutations are significant molecular biomarkers in cancer development and have been extensively studied for their prognostic relevance.⁵⁻⁷ The Ras gene family is

closely associated with tumorigenesis, with approximately one-third of tumors harboring Ras mutations. *TP53*, a key player in DNA damage repair and apoptosis, contributes to cancer development when mutated. Recent studies have explored *KRAS* and *TP53* mutations as potential targets for cancer therapy.⁸ Clinically, Zheng *et al.*⁹ found that colorectal cancer patients with *KRAS* mutations had significantly shorter survival. In another study involving 272 hepatocellular carcinoma patients treated with transarterial embolization, those with *TP53* mutations exhibited poorer survival outcomes.¹⁰

In the context of cholangiocarcinoma, current studies suggest that approximately 30% of patients carry *KRAS* mutations,¹¹ and sequencing of 382 cholangiocarcinoma cases revealed *TP53* mutations in 51.6% of patients.¹² Additional cholangiocarcinoma studies have also reported a strong correlation between *KRAS* or *TP53* mutation phenotypes and patient prognosis.^{13,14} However, despite these findings, the existing research does not yet provide definitive evidence on the specific mechanisms and prognostic impact of *KRAS* and *TP53* mutations in cholangiocarcinoma. Given this background, we believe that a meta-analysis of the available studies is necessary. Such an analysis will help integrate data from various studies and provide a comprehensive assessment of the prognostic significance of *KRAS* and *TP53* mutations in cholangiocarcinoma, thereby offering stronger scientific evidence for future clinical decisions and research directions. Through a comprehensive meta-analysis, we aim to elucidate the relationship between *KRAS* and *TP53* mutations and survival outcomes in cholangiocarcinoma, and further explore the potential of these genetic mutations as key molecular biomarkers for prognosis and treatment planning.

2. Materials and methods

A systematic and comprehensive literature search was conducted across several well-established public databases to identify relevant studies. The databases searched included PubMed, Web of Science, Cochrane Library, Embase, China National Knowledge Infrastructure (CNKI), and Wanfang Data, providing a broad representation of both international and Chinese research. The search was limited to studies published up to April 2025 to ensure the inclusion of the most up-to-date studies on *KRAS* and *TP53* mutations in cholangiocarcinoma. To ensure thorough coverage, the following search terms were used: “*TP53* gene” or “*KRAS* gene” combined with “cholangiocarcinoma” or “bile duct cancer,” further expanded to include “intrahepatic cholangiocarcinoma,” “cholangiocellular carcinoma,” “extrahepatic cholangiocarcinoma,” and “hilar cholangiocarcinoma.” These terms were then combined

with “survival,” “prognosis,” or “outcome” to identify studies specifically addressing the impact of these genetic mutations on patient outcomes. The search strategy was designed to be broad yet focused, aiming to capture all relevant studies that contribute to understanding the relationship between these mutations and survival in cholangiocarcinoma.

Two independent researchers conducted the literature search to minimize bias and ensure accuracy. Both researchers followed the same predefined search strategy, and the search was performed twice to confirm consistency. In addition, the reference lists of the included articles were manually reviewed to identify any additional studies not captured by the database search. This approach ensured that the literature search was exhaustive and comprehensive, capturing as many relevant studies as possible.

The inclusion criteria for studies are as follows: (1) studies investigating the relationship between *KRAS* and *TP53* gene mutations and prognosis in cholangiocarcinoma patients; (2) studies reporting survival outcomes, including odds ratios (ORs), hazard ratios (HRs), or sufficient data to compute these values; and (3) studies clearly identifying human populations and providing demographic information, such as age and sex, along with relevant clinical data. These criteria were designed to ensure the inclusion of studies with a focused scope and adequate detail for meaningful analysis.

Exclusion criteria were applied to ensure the quality and relevance of the selected studies. These criteria are as follows: (1) non-English or non-Chinese literature was excluded to ensure uniformity and the ability to assess studies based on widely recognized and accessible languages; (2) reviews, conference papers, case reports, clinical trials, animal studies, and cell/molecular studies were excluded as they did not directly address the prognosis of human cholangiocarcinoma patients harboring *KRAS* and *TP53* mutations; (3) studies lacking relevant survival data, such as OR, HR, or survival times, were excluded as these metrics are crucial for assessing the relationship between mutations and prognosis; (4) studies with a high risk of bias, such as those with unclear methodologies, incomplete data reporting, or inadequate control of confounding factors, were excluded to preserve validity; and (5) studies with short-term follow-up periods were excluded, as the analysis focused on long-term survival outcomes.

2.1. Data extraction and systematic evaluation

Data extraction was independently performed by two researchers following a standardized and rigorous protocol to ensure accuracy and clarity of all extracted data.

This systematic approach minimized human error and maintained the integrity of the meta-analysis by ensuring that data were comparable across the included studies. To maintain transparency and traceability, all information obtained during the data extraction process was archived, and a clear record was kept of each step taken. This archived data allowed for easy retrieval and verification of the information, thereby ensuring the robustness of the meta-analysis. In cases where disagreements or uncertainties arose during the data extraction process, they were resolved through consensus, with the involvement of a third reviewer to provide an objective resolution. This ensured that any potential biases were minimized, and all discrepancies were thoroughly addressed to maintain the accuracy of the data. The third reviewer acted as a neutral party, ensuring that decisions were made based on clear and consistent criteria.

The following data were extracted from the included studies: (1) the first author's name to identify the primary author and the study reference; (2) the year of publication to determine the recency of the study; (3) the country where the study was conducted to understand the geographic distribution of the study participants; (4) the number of patients included to gauge the sample size and statistical power of the study; (5) the race of the patient to identify any racial differences in the study population that could influence genetic variation and prognosis; (6) tumor location to determine whether intrahepatic, extrahepatic, or hilar cholangiocarcinoma impacted prognosis; (7) study design to classify the type of study (e.g., cohort or case-control) and to assess its potential bias and validity; (8) treatment methods to evaluate whether treatment regimens were consistent across studies; (9) detection methods for *KRAS* and *TP53* gene mutations; (10) the status *KRAS* and *TP53* genes (i.e., wild-type or mutated); (11) definitions of cutoff points to determine how thresholds for genetic mutations or prognostic factors were defined; and (12) follow-up time to ensure it was adequate to capture meaningful survival outcomes.

In addition, tumor clinical and pathological parameters were extracted, including tumor size, number of tumors, lymph node metastasis, distant metastasis, tumor differentiation, and TNM staging, as these are crucial for understanding cancer progression and prognosis. Survival outcomes such as overall survival (OS) or disease-free survival (DFS) were recorded as the primary endpoints for assessing the impact of genetic mutations on patient prognosis. In addition, the results of univariate and multivariate analyses for both OS and median survival were collected, including p-values, HRs, standard errors, and 95% confidence intervals (CIs). These metrics provide

critical insights into the strength of the association between mutations and prognosis.

The reliability of the included studies was further assessed by two researchers using the Newcastle-Ottawa Scale,¹⁵ a widely used tool for assessing the quality of non-randomized studies in meta-analyses. This scale evaluates three key areas of study quality: outcome assessment, comparability, and selection criteria. Outcome assessment included evaluating whether the inclusion and exclusion criteria were clearly defined, whether the follow-up period was sufficient to capture long-term outcomes, whether loss to follow-up significantly biased the study results, and whether outcome measurements for survival and disease progression were robust and valid. Comparability was assessed based on the availability of sufficient clinical data to support group comparisons, including patient characteristics such as age, sex, and pathological features. The studies were also evaluated on whether they were appropriately controlled for potential confounders, such as TNM staging and treatment methods, and whether the control groups were appropriately selected and analyzed. Selection criteria assessed the representativeness of the exposed cohort, considering whether the study population accurately reflected the broader population of cholangiocarcinoma patients. The selection of the unexposed cohort was also evaluated to determine whether it was comparable to the exposed cohort. We further assessed the scientific validity of the exposure factor analysis, verifying that *KRAS* and *TP53* mutations were correctly identified and properly attributed.

Each study was assigned a score based on these criteria, with a maximum possible score of 9. The total score was used to evaluate the methodological quality of each study and to ensure that only studies with sufficient rigor were included in the meta-analysis. This process enhanced the reliability and scientific rigor of the meta-analysis results, minimized the risk of bias, and strengthened the validity of the findings.

2.2. Statistical analysis

To assess the relationship between *KRAS* mutations, *TP53* mutations, and survival outcomes in cholangiocarcinoma patients, HRs and their corresponding 95% CIs were collected from each of the included studies. The HR is a commonly used measure to quantify the effect of a particular variable (in this case, *KRAS* or *TP53* mutations) on survival, with values >1 indicating an increased risk, and values <1 suggesting a protective effect. The 95% CI offers an estimate of the precision of the HR, with wider intervals indicating greater uncertainty.

To determine the consistency of the results across studies and to assess the heterogeneity, two standard statistical

tests were employed: the Q-test and the I^2 statistic. The Q-test tests the null hypothesis that all included studies share the same effect size. A $p > 0.05$ indicates no statistically significant heterogeneity, suggesting consistency among study findings, whereas a $p = 0.05$ or less indicates significant heterogeneity. I^2 statistic quantifies the percentage of total variation across studies that is due to heterogeneity rather than chance. An I^2 value $> 50\%$ is typically interpreted as moderate-to-high heterogeneity. In the presence of significant heterogeneity, defined by either a Q-test $p \leq 0.05$ or an I^2 value exceeding 50%, a random-effects model was applied for the meta-analysis. This model assumes that the true effect size varies between studies, accounting for both within-study and between-study variation. In contrast, when I^2 was $< 50\%$, indicating low heterogeneity, a fixed-effects model was used, which assumes a common effect size across all studies and provides a more precise estimate under such conditions.

To further evaluate the robustness of the results, a sensitivity analysis was performed. In this process, each study was sequentially removed from the analysis to observe the effect on the overall results. This helps identify any studies that may disproportionately influence the findings and allows for a more comprehensive assessment of the reliability of the meta-analysis conclusions. In addition, different statistical models were applied to assess whether the results were consistent across various methodological approaches, thus strengthening the validity of the conclusions.

Publication bias is a critical concern in meta-analyses, as studies with significant or positive results are more likely to be published, leading to an overestimation of the true effect size. To evaluate publication bias, both Egger's and Begg's tests were employed. These tests assess the symmetry of the funnel plot – a graphical representation of the relationship between study precision (typically the standard error) and effect size. A non-significant p -value (≥ 0.05) in either test suggests that there is no substantial publication bias, whereas a $p < 0.05$ indicates potential bias, possibly due to unpublished studies with negative or non-significant results, small sample sizes, or extreme findings. To correct for potential publication bias, the trim-and-fill method was applied. This statistical technique estimates the number of potentially missing studies that may be causing asymmetry in the funnel plot. It then adjusts the overall effect size by imputing these missing studies and recalculating the pooled estimate. This approach helps to correct for bias introduced by selective publication and enhances the robustness of the meta-analysis conclusions.

All statistical analyses were carried out using RevMan 5.4 (The Cochrane Collaboration, UK), Stata 17 (StataCorp

LLC, USA), and R version 4.2.3 (The R Foundation for Statistical Computing, Austria). These tools facilitated rigorous and transparent analysis of the data, ensuring the reliability and accuracy.

3. Results

3.1. Study selection and characteristics

This meta-analysis identified a total of 458 articles through systematic database searches, aimed at examining the relationship between *KRAS* and *TP53* gene mutations and survival prognosis in cholangiocarcinoma patients. After an initial screening of titles and abstracts, 76 articles were excluded due to duplication, and 324 articles were excluded because they did not meet the inclusion criteria based on the information available in their titles and abstracts. Subsequently, a more detailed assessment of the full-text articles led to the exclusion of an additional 18 articles due to insufficient data, which prevented them from contributing meaningfully to the analysis. Furthermore, 27 articles were excluded because their subject matter did not align with the focus of this meta-analysis, which specifically addressed the prognostic effects of *KRAS* and *TP53* mutations in cholangiocarcinoma. In addition, one article involving a clinical trial was excluded as clinical trials typically do not provide the same type of observational data necessary for meta-analysis. Ultimately, 12 studies were deemed eligible for inclusion, involving a total of 1,126 patients. Among these, 167 patients had *KRAS* mutations and 176 patients had *TP53* mutations, whereas 634 patients had *TP53* wild-type and 816 patients had *KRAS* wild-type. The studies included in the analysis were all retrospective cohort studies, reflecting a diverse array of patient populations and clinical settings. Retrospective cohort studies are valuable for understanding long-term outcomes and identifying associations between genetic mutations and survival in real-world clinical practice.

Regarding the geographic representation of the studies, six studies (50%) focused on Asian populations, whereas the remaining focused on European and American populations. Among the included studies, three involved patients who had received immunotherapy as part of their treatment regimen. Five of the studies focused specifically on patients with intrahepatic cholangiocarcinoma (ICC). In contrast, other studies included patients with extrahepatic (ECC) or hilar cholangiocarcinoma. Three studies involved patients who were receiving non-surgical treatments, and two studies included patients with advanced-stage disease (stage III and IV), which is associated with poorer outcomes. The inclusion of patients across different treatment modalities and disease stages contributed to the comprehensiveness of the analysis, allowing for a broader representation of clinical scenarios.

All 12 studies reported survival curves that allowed for the examination of the relationship between *KRAS* and *TP53* mutations and survival outcomes in cholangiocarcinoma. The survival curves were essential for understanding how these mutations influence patient prognosis. Among the included studies, five provided 95% CIs necessary for calculating HRs. The remaining studies provided sufficient data, including survival time and event data, to calculate HRs and 95% CIs. The specific characteristics of each of the 12 included studies are summarized in Table 1, outlining study designs, patient demographics, treatment regimens, and key findings related to the impact of *KRAS* and *TP53* mutations on survival outcomes. In addition, Figure 1 illustrates the study flow, illustrating the steps of the systematic review process from the initial database search to the final inclusion of studies. This visual representation enhances transparency and provides clarity regarding the selection and inclusion criteria applied in the meta-analysis.

3.2. Meta-analysis findings

This meta-analysis ultimately included 12 relevant cholangiocarcinoma studies. Among them, 10 studies described the relationship between *KRAS* gene status and patient survival, and five studies described the relationship between *TP53* gene status and patient survival. Our results indicate that patients with *KRAS* mutations have a poorer prognosis compared to those without mutations (HR=7.26; 95% CI: 6.10 – 9.81; $p < 0.05$), and no significant heterogeneity was found among the studies (fixed-effects model; $I^2 = 48\%$). The forest plot related to the *KRAS* study is shown in Figure 2, with a significant overall effect and a highly significant difference between *KRAS* mutant and

wild-type groups ($Z = 8.39$ and $p < 0.0001$). Further, Begg’s test indicated no publication bias ($p = 0.1286$), whereas Egger’s test indicated ($p < 0.05$). Therefore, we performed a trim-and-fill analysis, and the results are shown in Figure 3. The trim-and-fill method, a classic adjustment technique for publication bias, corrects for the asymmetry in the effect size distribution using an iterative trimming process. This procedure involves two stages: (1) the trimming stage (trim), where extreme effect size observations (often due to small-study effects or selective publication) are identified and excluded using non-parametric iterative algorithms; and (2) the filling stage (fill), where missing studies are simulated and added to restore symmetry in the funnel plot. In this study, we applied the trim-and-fill method using the R software, and the results showed that after adjustment, one potentially missing study was identified ($k^* = 1$), increasing the total number of studies to 11 ($kadj = 11$). As shown in Figure 3, after the adjustment, the analysis indicated a highly significant relationship between *KRAS* mutation and survival differences, with a statistically significant effect estimate of 12.84 units ($p < 0.0001$; 95% CI: 9.01 – 16.670). These results suggest that *KRAS* mutations play an important role in the survival prognosis of cholangiocarcinoma patients.

When the *TP53* gene status in cholangiocarcinoma prognosis was evaluated, our analysis showed that patients with *TP53* mutations have a poorer prognosis compared to those without mutations (HR=20.03; 95% CI: 6.79 – 33.28; $p < 0.05$). The analysis showed some heterogeneity (fixed-effects model: $I^2 = 59\%$; $p < 0.05$, overall effect 95% CI=11.24 – 25.32; random-effects model: $I^2 = 59\%$, $p < 0.05$, overall effect 95% CI: 6.79 – 33.28). The forest plot for *TP53* analysis is shown in Figure 4,

Table 1. Characteristics of the eligible studies

References	Country	KRAS (n)	TP53 (n)	Tumor location	Stage	Therapy	Survival analysis	Follow-up (month)	Cut-off (%)	NOS score
Boerner et al. 2021 ⁴⁴	USA, Netherlands	412	412	CCA	I-IV	Various	OS	60	5	8
Peng et al. 2022 ²⁵	China	12	/	ICCA	I-IV	No surgery	OS	20	5	7
Guo et al. 2022 ⁴⁵	China	26	/	CCA	I-IV	Various	OS	26.6	5	6
Ardito et al. 2022 ⁴⁶	Italy	54	/	ICCA	I-IV	Various	OS	180	5	8
Robertson et al. 2013 ⁴⁷	USA	52	/	CCA	II-IV	Various	OS	60	5	8
Javle et al. 2016 ⁴⁸	USA	224	224	ICCA	III-IV	Various	OS	58.3	5	8
Jin et al. 2023 ⁴⁹	China	37	/	CCA	I-III	Various	OS	50	5	8
Ikeno et al. 2018 ⁵⁰	Japan	50	/	CCA	I-IV	Various	OS	60	5	9
Conci et al. 2020 ⁵¹	Italy	99	103	CCA	I-IV	Various	OS	60	5	9
Chao et al. 2023 ⁵²	China	40	40	CCA	III-IV	No surgery	OS	24	5	9
Chen et al. 2012 ⁵³	China	83	/	ICCA	I-IV	Various	OS	108	5	9
Churi et al. 2014 ⁵⁴	USA	55	55	ICCA	I-IV	Various	OS	83.33	5	9

Abbreviations: CCA: Cholangiocarcinoma; ICA: Intrahepatic cholangiocarcinoma; NOS: Newcastle–Ottawa scale; OS: Overall survival.

showing a significant overall effect ($Z=5.09$) and a significant survival difference between the mutant and wild-type groups ($p<0.0001$). A subsequent sensitivity analysis (Figure 5) showed that removing individual studies did not result in statistically significant differences between studies, suggesting that the results were robust. After addressing publication bias, Begg's test and Egger's test yielded $p=0.806$ and 0.570 , respectively, indicating no publication bias ($p>0.05$).

4. Discussion

In the field of genomic research on cholangiocarcinoma, several studies have reported that *KRAS* and *TP53* mutations are more common compared to other genetic

alterations.^{11,16} The *KRAS* gene belongs to the RAS gene family, and through regulation of the RAF-MEK-ERK and PI3K-AKT-mTOR signaling pathways, it controls cellular growth and metabolism. Mutations in *KRAS* often lead to overactivation of downstream signaling pathways, thereby promoting tumor cell proliferation.¹⁷ On the other hand, mutations in *TP53* disrupt its ability to bind to its target DNA sequence, rendering its tumor-suppressive function ineffective.¹⁸

Current studies suggest that cancer patients with *KRAS* or *TP53* mutations have poorer survival outcomes compared to those without these mutations.^{19,20} In a meta-analysis involving stage II and III colon cancer patients, *KRAS* mutations were found to be associated with shortened DFS.²¹ Another meta-analysis on the prognostic role of *TP53* mutations in prostate cancer demonstrated that patients with *TP53* mutations had approximately 13%, 20%, and 16% lower OS at 1, 3, and 5 years, respectively, compared to patients without the mutation. *TP53* mutations were also associated with increased risk of death and accelerated disease progression.²²

In our study, the results indicated that cholangiocarcinoma patients with *KRAS* and *TP53* mutations had a poorer prognosis compared to those without mutations. This meta-analysis summarized 12 relevant studies, revealing that patients with *KRAS* mutations had worse prognoses compared to those without the mutation ($HR=7.26$; 95% CI: 6.10 – 9.81; $p<0.05$). Statistically, the survival differences between the *KRAS* mutant and wild-type groups were highly significant ($Z=8.39$; $p<0.0001$). This suggests that *KRAS* mutations, as an important genetic biomarker, may play a key role in the prognosis assessment of cholangiocarcinoma. No significant heterogeneity was found in the *KRAS* studies (fixed-effects model; $I^2=48\%$), supporting the use of a fixed-effects model for further analysis. However, Egger's test

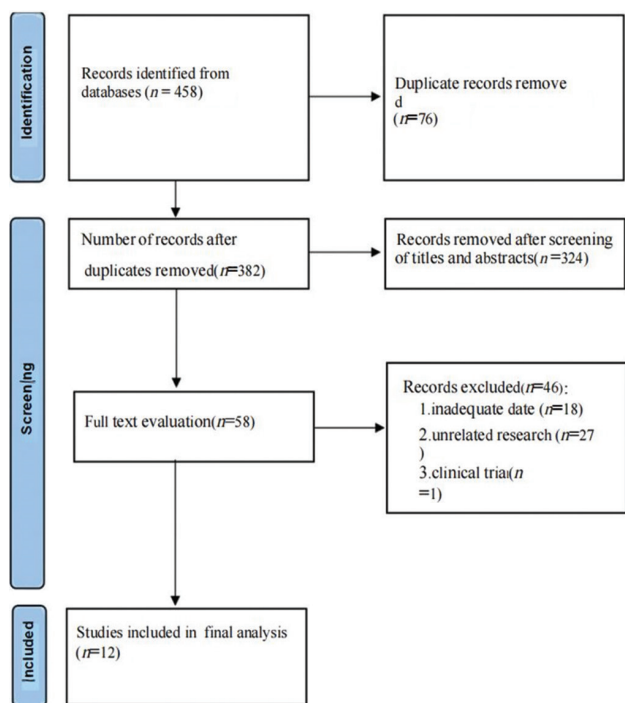


Figure 1. Flow chart of the study design

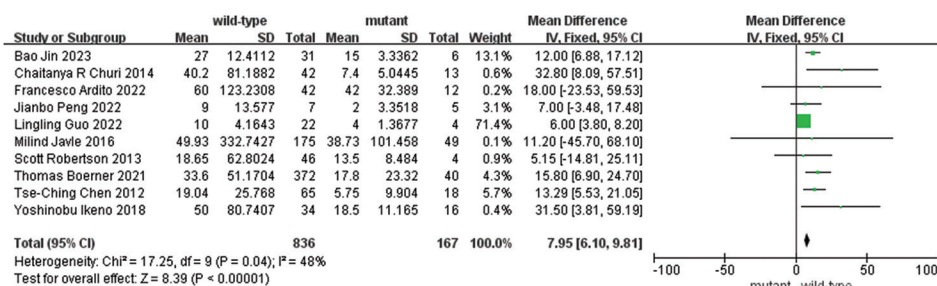


Figure 2. Forest plot comparing OS between mutant and wild-type *KRAS* in cholangiocarcinoma patients. The size of each square represents the weight of the corresponding study in the meta-analysis, whereas the horizontal lines indicate 95% confidence intervals. The pooled estimate is represented by the diamond at the bottom.

Note: The inclusion of these green marks is intended to draw attention to key findings that are of particular interest in the context of the meta-analysis. Abbreviations: CI: Confidence interval; df: Degrees of freedom; IV: Inverse variance; OS: Overall survival; SD: Standard deviation.

($p < 0.05$) suggested potential publication bias, indicating that some negative or small sample studies may not have been included in the analysis. Therefore, we applied the trim-and-fill method to correct for publication bias. After the correction, we found that one study was missing from the original analysis; adding it increased the number of studies from 10 to 11, further confirming the significant relationship between *KRAS* mutations and survival differences (effect estimate=12.84 units, $p < 0.0001$). This corrected result further strengthened our conclusions.

In the analysis of *TP53* gene mutations, we found that patients with *TP53* mutations had significantly poorer prognoses compared to those without mutations (HR=20.03; 95% CI: 6.79 – 33.28; $p < 0.05$). The adverse prognostic effect of *TP53* mutations in cholangiocarcinoma was further statistically supported ($Z=5.09$; $p < 0.00001$). We observed some heterogeneity in the *TP53* studies ($I^2=59\%$; $p=0.04$), and therefore, a random-effects model was used for the analysis. Nevertheless, sensitivity analysis

showed that excluding individual studies did not lead to statistically significant differences, indicating that our conclusions were robust. Further testing for publication bias using Begg's test ($p=0.806$) and Egger's test ($p=0.570$) showed no significant publication bias, further validating the reliability of our results.

In this study, we focused on analyzing the relationship between *KRAS* mutations, *TP53* mutations, and survival prognosis in cholangiocarcinoma patients. Although mutations in *KRAS* or *TP53* alone are associated with poorer survival outcomes, increasing evidence suggests that the co-occurrence of *KRAS* and *TP53* mutations may have a more pronounced effect on patient prognosis. Previous studies have indicated that, in various cancer types, patients with co-mutations of *KRAS* and *TP53* often exhibit more aggressive tumor behavior, poorer treatment response, and shorter survival, especially in malignant cancers such as pancreatic and colorectal cancer.^{23,24} This could be due to the dual mutations, which not only exacerbate tumor proliferation but also impair cellular self-repair mechanisms.

While individual *KRAS* or *TP53* mutations have been widely studied in cholangiocarcinoma, systematic and large-scale investigations into the biological and clinical impact of their co-mutation remain limited. Among the studies included in our meta-analysis, one reported a *KRAS* and *TP53* co-mutation rate of 16.7% in patients with ICC.²⁵ However, due to its limited sample size, the study did not compare survival outcomes between patients with co-mutations and those with only a single mutation. To address this gap, future studies should further explore the specific role of *KRAS* and *TP53* co-mutations in survival prognosis, treatment response, and drug resistance in cholangiocarcinoma patients through multi-center, large-sample cohort studies. In addition, combining clinical data with molecular biological analyses to reveal how the co-mutation of these two genes coordinates at the molecular level will provide more precise evidence for early diagnosis, prognosis evaluation, and personalized

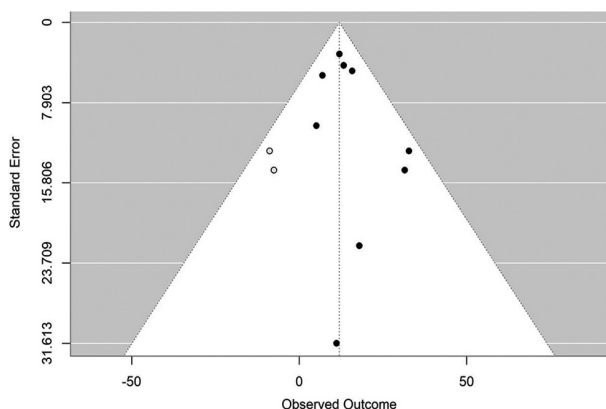


Figure 3. Data trimming outcome for *KRAS* analysis. The black circles represent actual studies included in the analysis, while the white circles indicate studies that were imputed using the trim-and-fill method. The trim-and-fill method is used to adjust for potential publication bias by estimating and adding missing studies to balance funnel plot asymmetry. This adjustment provides a more accurate reflection of the overall effect size and helps to correct for publication bias in the analysis.

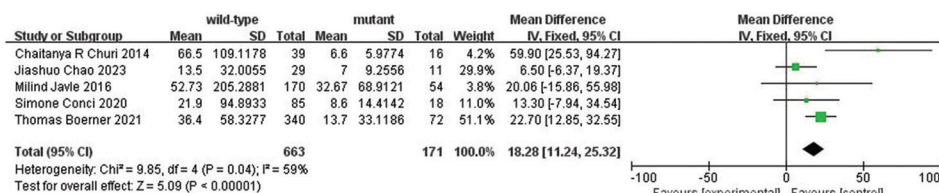


Figure 4. Forest plot comparing OS between mutant and wild-type *TP53* in cholangiocarcinoma patients. The size of each square represents the weight of the corresponding study in the meta-analysis, while the horizontal lines indicate 95% confidence intervals. The pooled estimate is represented by the diamond at the bottom.

Note: The inclusion of these green marks serves to direct attention to key findings that are of particular interest in the context of the meta-analysis. Abbreviations: CI: Confidence interval; df: Degrees of freedom; IV: Inverse variance; OS: Overall survival; SD: Standard deviation.

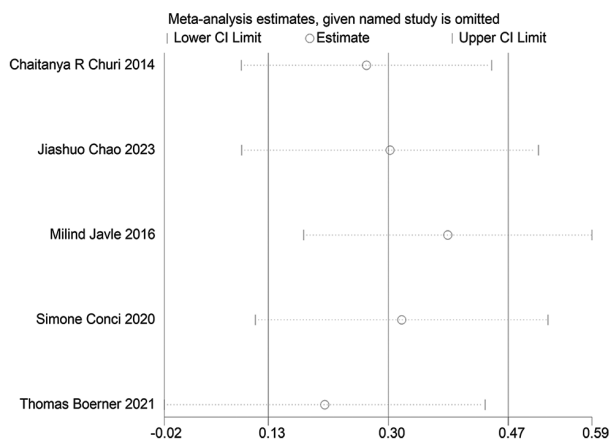


Figure 5. Sensitivity analysis of *TP53* studies
Abbreviation: CI: Confidence interval.

treatment of cholangiocarcinoma.

It is noteworthy that in the treatment of cholangiocarcinoma, immunotherapy – as an emerging therapeutic approach – has shown promising results.^{26,27} *KRAS* and *TP53* mutations, two common oncogenic drivers, have been shown to influence immune evasion mechanisms in various cancer types, which may significantly affect responses to immunotherapy.²⁸⁻³⁰ Several studies have demonstrated that *KRAS* and *TP53* co-mutations are associated with a significant increase in PD-L1 expression, along with higher tumor burden and immune cell infiltration.^{31,32} Two studies on lung adenocarcinoma found that patients with *KRAS* and *TP53* co-mutations responded better to immune checkpoint inhibitors.^{33,34} In cholangiocarcinoma, Chen *et al.*³⁵ observed differing clinical benefits between patients with only *KRAS* mutations and those with both *KRAS* and *TP53* co-mutations receiving immunotherapy. Specifically, patients with *KRAS* mutations alone exhibited poor responses to immunotherapy, whereas those with *KRAS-TP53* co-mutations demonstrated elevated PD-L1 expression and greater immune cell infiltration. Among all genetic subgroups, cholangiocarcinoma patients with various combinations of *KRAS* and *TP53* mutations achieved the highest objective response rates following immunotherapy.³⁵

In our study, we analyzed the relationship between *KRAS* and *TP53* mutations and survival prognosis in cholangiocarcinoma patients. However, with the advancement in molecular biology, growing evidence suggests significant differences in the mutation profiles of various cancer subtypes, which may influence the clinical applicability of prognostic models. For instance, a study found that the prognostic value of TROP2 varies

by lung cancer subtype.³⁶ Similarly, different subtypes of cholangiocarcinoma – such as ICC and ECC – exhibit distinct genetic mutation profiles. ECC patients are more likely to harbor *KRAS* and *TP53* mutations, whereas ICC patients more commonly present with *IDH1* mutations. These differences may reduce the predictive accuracy of unified prognostic models across subtypes.^{37,38} Therefore, developing subtype-specific prognostic models based on unique mutation profiles may significantly enhance clinical applicability and accuracy. This has become an active area of research.^{39,40} Although our study employed a unified prognostic model, recognizing the different roles that *KRAS* and *TP53* mutations play in each subtype highlights the need for subtype-specific tools. A one-size-fits-all approach may overlook meaningful distinctions, whereas subtype-tailored models could offer more accurate, personalized prognostic assessments.

Due to factors such as liver fluke infection and consumption of undercooked fish, regions including northeastern Thailand, Korea, and Japan are considered high-risk areas for cholangiocarcinoma.⁴¹⁻⁴³ However, because of the limited number of eligible studies from these regions, they were not included in our analysis. While the higher incidence rates in these areas did not conflict with our search criteria, the lack of regional representation may affect the comprehensiveness of our findings. Future research should prioritize incorporating data from these high-risk regions to further validate the prognostic significance of *KRAS* and *TP53* mutations. In addition, due to the insufficient number of eligible studies, we were unable to conduct subgroup analyses based on treatment modalities (e.g., chemotherapy and immunotherapy) or mutation types (*KRAS* only, *TP53* only, or co-mutations). Furthermore, data limitations prevented adjustments for confounding variables such as tumor location (hilar, intrahepatic, or extrahepatic) or stage. Finally, this study only included published literature, excluding unpublished studies with negative results. Future investigations are needed to validate and expand upon the prognostic effects of *KRAS* and *TP53* mutations in cholangiocarcinoma patients.

5. Conclusion

Cholangiocarcinoma is a highly heterogeneous malignant tumor, with *KRAS* and *TP53* gene mutations playing a crucial role in its occurrence and development. Our findings indicate that mutations in *KRAS* and *TP53* are each independently associated with poor prognosis in cholangiocarcinoma patients. However, existing studies exhibit certain biases and heterogeneity. Therefore, further high-quality research is essential to validate and refine these conclusions, ultimately contributing to more

effective and individualized treatment strategies for cholangiocarcinoma.

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Conflict of interest

The authors declare no conflicts of interest.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Data are available from the corresponding author upon reasonable request.

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