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Association of the K-RAS Gene Mutation with Gallbladder Cancer

Jesteger Eliseo Hernández–Vázquez[1] and Keila Concepción-Xicoténcatl[2]*

[1]*Instituto mexicano del seguro social delegación estatal Chiapas coordinación de educación e investigación en salud hospital general de zona no. 2, Servicio de Cirugia general. Tuxtla Gutiérrez, Chiapas, Mexico*

[2]*Instituto de Seguridad y Servicios Sociales de los Trabajadores del Estado, Hospital General "Dr. Daniel Gurria Urgell", Servicio de Anatomía patológica. Villahermosa Tabasco, Mexico*

***Corresponding author:** Keila Concepción-Xicoténcatl, Instituto de Seguridad y Servicios Sociales de los Trabajadores del Estado, Hospital General "Dr. Daniel Gurria Urgell", Servicio de Anatomía patológica. Villahermosa Tabasco, Mexico

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ABSTRACT

The early development of gallbladder cancer is usually asymptomatic and has a high tendency to metastatic spread, so most patients are diagnosed in intermediate to advanced stages. The role of various genetic mutations is a currently active field of research that has sometimes transformed the diagnosis and/or treatment of some types of cancer. The point mutation in codon 12 of the K-ras gene has become the target of analysis in different studies, because there is considerable debate about the frequent presence of this mutation in malignant lesions of the gallbladder. Therefore, demonstrating whether there is a significant association through an analytical study could be useful to offer a useful molecular diagnostic marker in the detection of the early stage of carcinogenesis in the gallbladder and reduce a public health problem. This meta-analysis was performed through a systematic search using the PubMed and EBSCO library databases, where 10 studies with a total of 221 cases and 163 controls were selected. Publication bias was assessed using the GRADE approach and the quality of each study was assessed independently using the Newcastle-Ottawa Assessment Scale. Epi-data version 3.1 software was used to assess this association, where our results demonstrated a significant association between the mutation of codon 12 of the K-ras gene and the risk of gallbladder cancer (OR: 0.18; IC 95%: 0.08-0.41). The P value of the Q test was: 0.32.

Keywords: Gallbladder; K-ras; Genetic mutations

Introduction

Gallbladder cancer (GBC) is the most common cancer of the biliary tract [1]. Early development of gallbladder cancer is usually asymptomatic and has a high propensity for metastatic spread [2]. BVV incidences vary by geographic location and ethnicity. In fact, the highest rates have been found among North American Indian, Mexican American, Bolivian, Chilean, Central European, and northern Indian populations [3]. GBC is one of the most aggressive malignant tumors and has a high lethality (median overall survival at 5 years of 18%) [4]. According to world cancer statistics in the year 2020, 115,949 people have been diagnosed with GBC worldwide and 84,965 have died from this condition [5]. In almost all countries, it is frequently diagnosed during advanced stages, which limits therapeutic options and results in a poor prognosis [6]. Gallbladder cancer arises from a premalignant intraepithelial lesion. The definition and classification is ambiguous and studies on its clinical features have not been actively conducted. Due to the skewed prevalence throughout the world, there is little interest in research and information on its molecular biology is lacking [7]. The etiology of GBC is complex and multifactorial. Several risk factors associated with its development include geographic distribution, genetic susceptibility, race, advanced age, gender, gallstones (size > 3 cm), and chronic inflammation [8]. Models of gallbladder carcinogenesis have recently been shown to suggest that it affects a number of genetic and epigenetic events during the initia-

tion and progression of malignancy. While the precise nature of all the steps leading to malignancy remains unknown, several high-throughput studies have reported a complex interplay of genomic, epigenomic, transcriptomic, and proteomic profiling [9].

Surgery is the only potentially curative treatment, but it is only possible for the minority of patients diagnosed with GBC. However, recent advances include specific therapies for the most common genetic alterations. These new therapeutic targets offer the opportunity for more personalized therapy for GBC patients in the future [10]. K-ras is one of the most common mutated oncogenes in cancer. This gene is located on chromosome 12p12.1 and is approximately 38 kilobases (kb) in length with four exons 11. In particular, point mutations in codon 12 are present in up to 80% of KRAS mutant malignancies, of which 41% are G12D (GGT to GAT mutation substituting glycine 12 for aspartic acid), 28 % are G12V (mutation from GGT to GTT that replaces glycine 12 with valine) and 14% are G12C (mutation from GGT to TGT that replaces glycine 12 with cysteine) 12. This mutation causes effects in a change of the protein to the activated state, the mutagenic activates the signal transduction pathways [11]. It is believed that the K-ras proto-oncogene exerts control over some of the growth and differentiation mechanisms in the development of gallbladder cancer [12], and recent studies have shown that there is a frequency of this mutation in relation to CVB in a percentage of 0 % to 41% [13]. Although several studies of codon 12 mutation of the K-ras gene in patients with gallbladder cancer have been published. A systematic review and meta-analysis evaluating this association are lacking.

Methodology

Search Strategy and Identification of Relevant Studies

The protocol for this meta-analysis was registered with PROSPERO, registry number CRD42023418115. A comprehensive search of electronic databases, including PubMed and EBSCO, was performed to identify relevant publications reporting the association between codon 12 mutation of the K-ras gene in patients with gallbladder cancer, and the last search was updated on April 17, 2023. Relevant studies were identified using the terms: "K-RAS and GBC", "K-RAS codon 12 mutation with Gallbladder cancer". References within retrieved articles and review articles were also examined. The citation lists of the retrieved articles were manually examined to ensure the sensitivity of the search.

Inclusion and Exclusion Criteria

Inclusion criteria were:

1. Case-control studies investigating the association between codon 12 mutation of the K-ras gene and CVB risk,
2. To be published in peer-reviewed journals,
3. That contained independent data,
4. The articles had to be written in English and Spanish,
5. That mutations in codon 12 of the K-ras gene were detected by polymerase chain reaction (PCR).

The exclusion criteria were:

1. Duplicate publications,
2. Studies that were not case/control;
3. Abstracts, commentaries, reviews, congressional proceedings or editorials and
4. Insufficient data reported.

Data Extraction

All available data was extracted from each study according to the inclusion criteria listed above. Data such as authors, year of publication, study population, number of cases and controls, GBC diagnosis of the participants, and mutations detected in both analysis groups were taken into account.

Quality Score Evaluation

The quality of each study was assessed independently using the Newcastle-Ottawa Assessment Scale (NOS) for inclusion in the systematic review by estimating methodological quality. The quality score for a given study was based on a score of 6 as the cut-off point to distinguish high-quality from low-quality studies.

Publication Bias

The presence of possible publication bias was assessed graphically using funnel plot drawing and statistically using Egger's standard regression test. In Egger's test, $p<0.05$ was considered statistically significant publication bias. In addition, to strengthen the analysis, publication bias and risk were assessed using the GRADE approach. Also, the 95% confidence interval (95% CI) was calculated; where, an effect size of 0.2 was considered small, an effect size of 0.5 was considered moderate, and >0.8 was considered large.

Statistic Analysis

For statistical analysis, the Epi-data version 3.1 software was used. Results are presented as odds ratio (OR) and are used to assess the association of the K-ras gene codon 12 mutation with the risk of gallbladder cancer. The heterogeneity of the sample was analyzed using the Dersimonian and Laird Q test. The results of the Q test were supplemented with graphs to help visualize those studies that favor heterogeneity.

Results

Study Characteristics

The PRISMA16 flowchart is shown in Figure 1. A total of 764 records were obtained by searching the PubMed and EBSCO databases. After removing the duplicates, we found 24 potentially relevant records. When reviewing titles, abstracts, and full texts according to the inclusion and exclusion criteria established in this study, 754 articles were excluded because they did not have sufficient data of interest. Finally, 10 full-text articles met our inclusion criteria and were included in the final analysis [14-16].

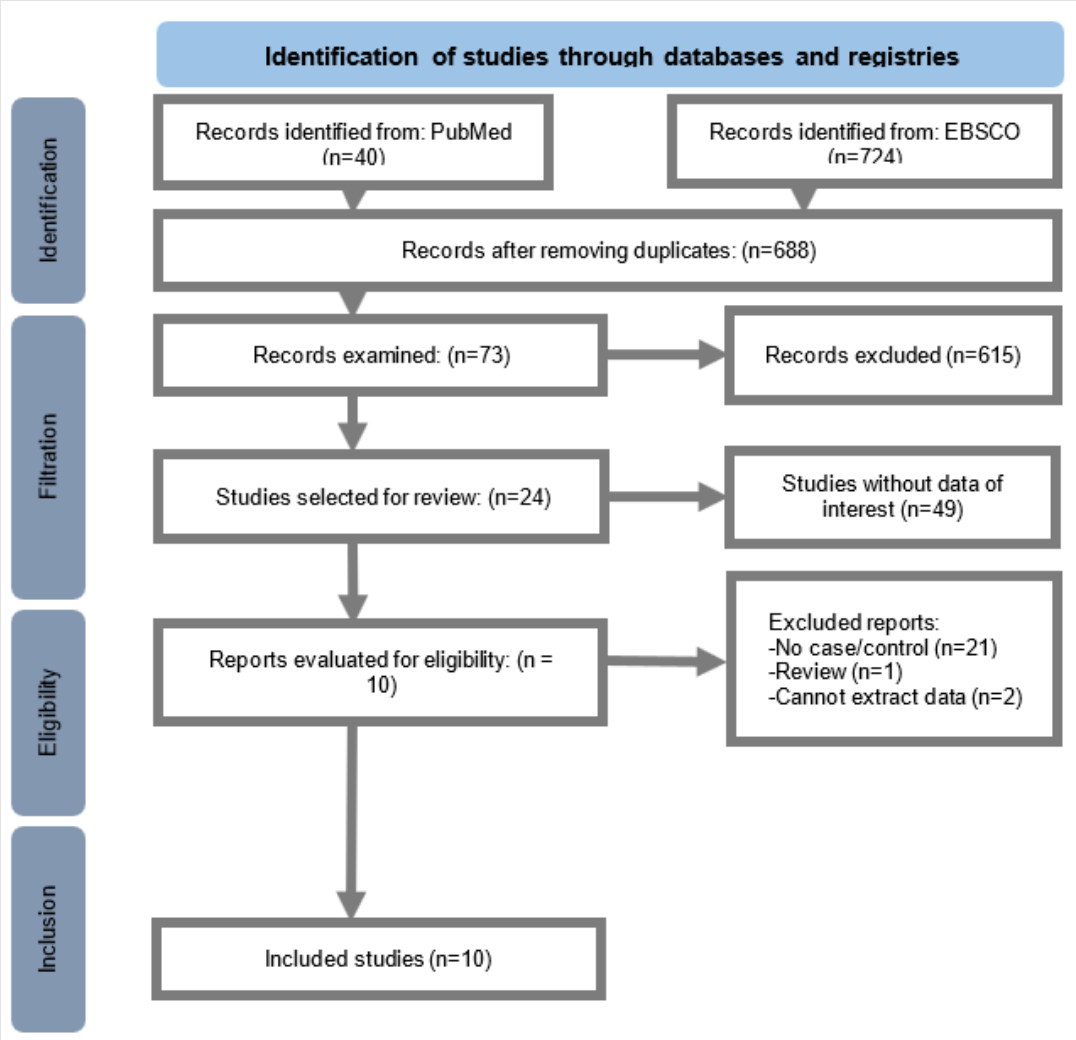

Figure 1: Design of flowcharts for the inclusion of studies in this meta-analysis.

Data Extraction and Quality Assessment

The reviewed articles were conducted between the years 1996 to 2019. The general characteristics of all eligible studies are summarized in Table 1 [17-26]. Quality details based on Newcastle Ottawa scale score assessment for each study is shown in Tables 1 & 2.

Table 1: Summary of the findings of the association studies on the mutation of codon 12 of the K-ras gene in the risk of CVB.

Referencia	Número de pacientes (casos/controles)	Población	Gen K-ras No mutado Casos	Gen K-ras Mutado Casos	Gen K-ras No mutado Controles	Gen K-ras Mutado Controles	Valor de P
Ajiki, et al. [17]	51/10	Japón	21 (41.18%)	30 (58.82%)	10 (100%)	0	0.0054
Itoi, et al. [18]	20/6	Japón	18 (90%)	2 (10%)	6 (100%)	0	0.0058
Matsubara, et al. [19]	5/3	Japón	1 (20%)	4 (80%)	3 (100%)	0	0.1967
Iwase, et al. [20]	11/11	Japón	7 (63.64%)	4 (36%)	11 (100%)	0	0.1316
Ito, et al. [21]	3/5	Japón	2 (66.67%)	1 (33.33%)	5 (100%)	0	0.7469
Tanno, et al. [22]	6/6	Japón	2 (33.33%)	4 (66.67%)	6 (100%)	0	0.0943
Hanada, et al. [23]	8/4	Japón	5 (62.50%)	3 (37.50%)	4 (100%)	0	0.5967
Itoi, et al. [24]	46/45	Japón	27 (58.70%)	19 (41.30%)	45 (100%)	0	0.0058
Kazmi, et al. [25]	39/24	India	23 (58.97%)	16 (41.03%)	23 (95.83%)	1 (4.16%)	0.0056
Tomioka, et al. [26]	32/49	Japón	27 (84.38%)	5 (15.63%)	45 (91.83%)	4 (8.16%)	0.4946

Table 2: Methodological quality of the studies included in this meta-analysis based on the Newcastle Ottawa scale score.

Referencia	Selección	Comparabilidad	Exposición	Puntuación total
Ajiki, et al. [17]	★★★	★	★★★	7
Itoi, et al. [18]	★★★	★	★★★	7
Matsubara, et al. [19]	★★★	★	★★★	8
Iwase, et al. [20]	★★★★	★	★★★	8
Ito, et al. [21]	★★★	★	★★★	8
Tanno, et al. [22]	★★★★	★	★★★	8
Hanada, et al. [23]	★★★	★	★★★	7
Itoi, et al. [24]	★★★	★	★★★	7
Kazmi, et al. [25]	★★★	★	★★★	8
Tomioka, et al. [26]	★★★	★	★★★	8

Publication Bias

Begg's test was performed to quantitatively assess the publication bias of the included studies. The shape of the funnel plots (P value 0.47; Z statistic: 0.71), and this was confirmed with the Egger test (P value: 0.87), in the same way the analysis of heterogeneity is represented by the Galbrait plot. these results are shown in Figure 2. Detailed information for the publication bias test is shown in Table 3.

Table 3: Summary of the results of association studies on the K-ras 12 codon mutation and GBC risk.

Reference	Number of atients	Study design	Odds Ratio (95% CI)	publication bias	Quality GRADE
Ajiki, et al. [17]	61	Case-control	0.21(0.09-0.49)	Not detected	Moderate
Itoi, et al. [18]	26	Case-control	0.16(0.07-0.34)	Not detected	Moderate
Matsubara, et al. [19]	8	Case-control	0.19(0.082-0.45)	Not detected	Moderate
Iwase, et al. [20]	22	Case-control	0.18(0.078-0.45)	Not detected	Moderate
Ito, et al. [21]	8	Case-control	0.17(0.077-0.41)	Not detected	Moderate
Tanno, et al. [22]	12	Case-control	0.19(0.084-0.46)	Not detected	Moderate
Hanada, et al. [23]	12	Case-control	0.17(0.074-0.39)	Not detected	Moderate
Itoi, et al. [24]	91	Case-control	0.24(0.11-0.52)	Not detected	High
Kazmi, et al. [25]	63	Case-control	0.22(0.096-0.50)	Not detected	Moderate
Tomioka, et al. [26]	81	Case-control	0.14(0.061-0.33)	Not detected	High

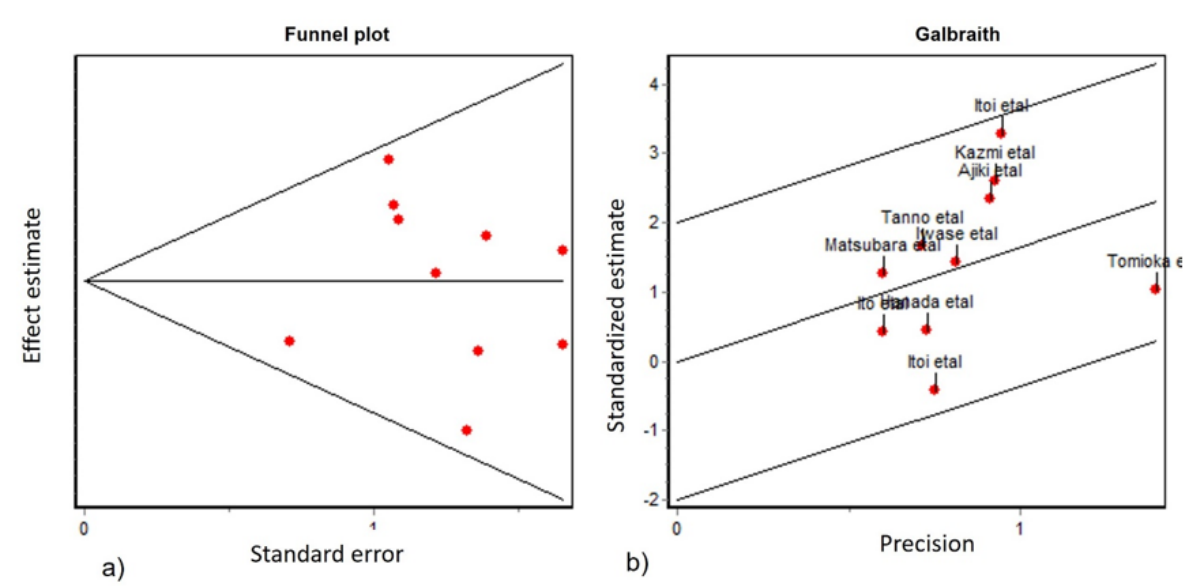

Figure 2: Funnel Plot and Galbraith Plot.

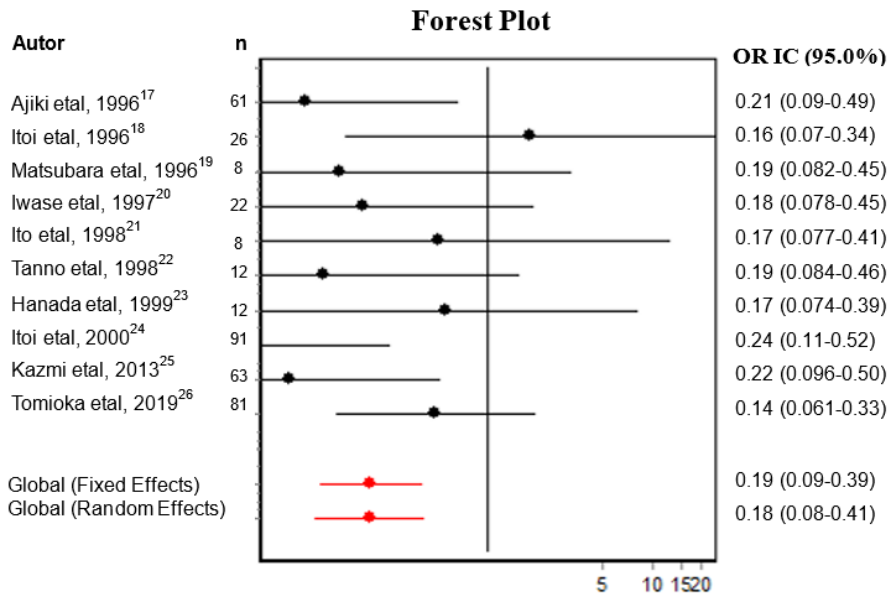

Figure 3: Forest Plot.

General Analysis

Figure 3 shows the main results of the meta-analysis. These showed a significant association with the risk of GBC (OR: 0.18; 95% CI: 0.08-0.41). The P value of the Q test was: 0.32.

Discussion

Gallbladder cancer is the most common malignant tumor of the bile ducts, accounting for 80% to 95% of malignant bile duct tumors. The overall median survival of patients with GBC is only 6 months, and the 5-year survival rate is <5%27. The literature has described genes encoding various types of proteins that help control cell growth and proliferation. It is widely known that gene mutations can contribute to the development of tumors28. The general prognosis of GBC is poor and there is an imminent need for further therapeutic intervention in addition to radical surgeries. Therapy directed at molecular receptors that are associated with tumor invasion, proliferation, and immune response in multiple cancer types shows better survival29. Therefore, the most widely studied genetic abnormality in gallbladder carcinogenesis is the K-ras gene mutation. Where, it has been reported that the frequency of the codon 12 mutation has been detected in gallbladder carcinoma; however, these data vary widely, from 0 to 100%, which can be attributed to the use of different sensitivities of the assay techniques used, or to racial and geographic variations in the populations studied30. In this meta-analysis, where 10 studies were included to analyze the association between codon 12 mutations of the K-ras gene in patients with GBC vs control patients, we obtained results that demonstrated a significant association between codon 12 mutation of the K-ras gene and GBC risk, where the codon 12 mutation frequency was higher in patients with GBC compared with the control group. Similarly, a previous study by (Roa, et al. [1]) found mutations in codon 12 of the K-ras gene in 30% of GBC cases;(Shukla, et al. [13]) also analyzed 25 patients with adenocarcinoma in the gallbladder, finding a 48% incidence of codon 12 mutation.

These results are consistent with our analysis. However, the results of (Singh, et al. [14]) suggest that the frequency of codon 12 mutations of the K-ras gene were not significantly associable in patients with GBC. Although we found a significant association in this relationship, there are several limitations to this meta-analysis [15-22]. First, gallbladder cancer may be influenced by variable confounders, such as geographic region, tumor stage, or other underlying genetic mutations. Second, there may be a linguistic bias because only studies published in English and Spanish were included [23-31]. Third, insufficient information about age, gender, and other potential confounding factors may affect our results. Given the limitations of the studies included in this meta-analysis, studies with larger sample sizes with prospective designs may be needed to fully understand the relationship. However, this analysis still provides new insights into the role of the codon 12 mutation in gallbladder cancer risk.

Conclusion

In conclusion, the present systematic review and meta-analysis showed a relationship between codon 12 mutation of the K-ras gene and the risk of GBC, suggesting that this could be a potential marker for gallbladder cancer.

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Keila Concepción-Xicoténcatl. Biomed J Sci & Tech Res

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