Research Article

A Systematic Review and Meta-analysis on the Occurrence of Biomarker Mutation in Colorectal Cancer among the Asian Population

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Received 19 January 2022; Accepted 24 May 2022; Published 23 June 2022

Academic Editor: Syed Sameer Aga

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Globally, colorectal carcinoma (CRC) is the third most common cancer and the third major cause of cancer-related death in both sexes. KRAS and BRAF mutations are almost mutually exclusively involved in the pathogenesis of CRC. Both are major culprits in treatment failure and poor prognosis for CRC. Method. A systematic review and meta-analysis of various research was done following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines. This trial is registered with PROSPERO CRD42021256452. The initial search included 646 articles; after the removal of noneligible studies, a total of 88 studies was finally selected. Data analysis was carried out using OpenMeta Analyst and Comprehensive Meta-Analysis 3.0 (CMA 3.0) software to investigate the prevalence of KRAS and BRAF mutations among patients with CRC in Asia. Results. The meta-analysis comprises of 25,525 sample sizes from Asia with most being male 15,743/25525 (61.7%). Overall prevalence of KRAS mutations was (59/88) 36.3% (95% CI: 34.5-38.2) with $I^2 = 54.5\%$ ($P$ value $< 0.001$). In 43/59 studies, frequency of KRAS mutations was majorly in codon 12 (76.6% (95% CI: 74.2–78.0)) and less in codon 13 (21.0% (95% CI: 19.1-23.0)). Overall prevalence of BRAF mutations was 5.6% (95% CI: 3.9-8.0) with $I^2 = 94.00\%$ ($P$ value $< 0.001$). When stratified according to location, a higher prevalence was observed in Indonesia (71.8%) while Pakistan has the lowest (13.5%). Conclusion. Total prevalence of KRAS and BRAF mutations in CRC was 36.6% and 5.6%, respectively, and the results conformed with several published studies on KRAS and BRAF mutations.

1. Introduction

Globally, cancer is a serious medical burden, and it is one of the main causes of death and morbidity throughout the world [1, 2]. With more than 1.8 million new CRC diagnoses and 0.86 million deaths globally in 2018 [3], colorectal carcinoma (CRC) is the third most common cancer and the third major cause of cancer-related death in both sexes [4]. The occurrence of CRC differs globally; the overall highest incidence rates of CRC may be seen in the United States, Canada, Europe, and Australia, whereas the lowest rates can be found in South-Central Asia and Africa [2]. However, the prevalence is rising exponentially in Asia [5], especially as the number of new cases of CRC is rapidly growing in Asia-Pacific thus, accounting for more than half of all new cases diagnosed globally [5, 6]. CRC prevalence rates vary
due to several factors such as ethnicity, genetics, regions, and lifestyles. It is reported to be 38 percent among Caucasians, 40 percent among Asians, and just 21 percent among Africans [6]. Pathogenesis of CRC involves gene mutation, mostly involving the MAPK-ERK cascade, for which the KRAS and BRAF are exclusively involved.

Nation-wise, the prevalence of KRAS and BRAF mutation varies regionally, and this is majorly due to genetic changes from heterogeneously related races [6]. From the World Health Organization (WHO) regional grouping, the prevalence of KRAS mutation among constituting nations with CRC is 30.23%, 35.12%, 31.83%, 33.17%, and 32.64% for the EMRO, EURO, PAHO, SEARO, and WAPRO, respectively, [5]. Colorectal carcinogenesis is a multisignalling process involving four major pathways: the Wnt-β catenin pathway, MAPK/ERK pathway, PI3K/Akt pathway, and p53 pathway. Each pathway involves several sequential genetic modifications, such as chromosomal anomaly, gene mutations, and/or epigenetic changes, that turn normal colonic epithelium into colorectal cancer [7] [8]. Like the KRAS gene, BRAF is also part of the Ras family that targets the RAS/RAF/MEK/ERK pathway; together, they both account for 7-25% and 5-20% of all cancers as well as 30-45% and 8-10% of CRC for both KRAS and BRAF, respectively [8, 9]. Mutations in KRAS and BRAF are almost mutually exclusive. The detection or testing for KRAS and BRAF gene mutation presents a blueprint and change to standard diagnostic guidelines for inpatient care and creates a major development in early decision-making in personalizing cancer care. The identification of this mutation would be crucial for the prognosis of CRC patients. Early diagnosis and treatment will improve patients’ standard of health, increase their chances of survival, and lower morbidity and mortality. The poor prognosis of metastatic CRC has fuelled continued efforts to identify therapeutic options that will improve patient outcomes via detailed gene profiling such as in KRAS and BRAF mutations.

Ras proteins are tiny guanosine triphosphatases (GTPases) to which the KRAS and BRAF genes belong. Through the GTPase cascade, they govern a variety of intracellular activities such as proliferation, differentiation, immune response, and survival rate [10]. The understanding of genetic alterations (such as in KRAS and BRAF mutations) in metastatic CRC (mCRC) via the use of gene profiling can be a catch point in explaining the gene’s resistance to antipidermal growth factor receptor (EGFR) antibody management [11] and as a prognostic predictor in arresting the progress of CRC [12] [8, 13]. Both KRAS and BRAF are downstream EGFR oncogenes, for which their mutations can activate EGF receptor signalling in cancer cells and are linked with poor prognosis in the CRC. Hence, certain aberrations or mutations that have a well-established prognostic and predictive blueprint in CRC are now regularly examined as a component of clinical therapy [14]. Through this study, the authors intend to determine the prevalence of KRAS and BRAF gene mutations in CRC and whether the prevalence of mutated KRAS and mutated BRAF genes in colorectal carcinoma differs among patients in Asia via literature review and meta-analysis to provide a very accurate KRAS and BRAF mutation estimates.

2. Materials and Methods

The present study is a systematic review and meta-analysis of various researched and published papers that were carried out following the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) [15]. The study protocol was registered with PROSPERO (registration number: CRD42021256452).

2.1. Literature Search and Selection Criteria. In the study, articles were retrieved from three electronic databases (PubMed, Scopus, and ScienceDirect); the eligible studies were searched and assessed using a combination of relevant keywords: (“colorectal cancer” OR “colon cancer” OR “metastatic colon cancer” OR “metastatic colorectal cancer” OR “CRC” OR “Rectum”) and (“BRAF” OR “BRAF” OR “c-BRAF” OR “KRAS” OR “K-RAS” OR “c-KRAS”) and (“Asia”). The full details of the search strategies for this study are in the supplementary search strategic file. A comprehensive search for the most relevant studies was carried out by combing through titles, keywords, and abstracts of various papers. The initial search included 646 articles (Figure 1) which were performed on 10 April 2021 via the EndNote X9 software; references of all assessed studies were exported to the software after which, duplications were then removed. The inclusion criteria for the studies selected for this meta-analysis include cross-sectional studies, cohort studies, or case series carried out to investigate the frequency of KRAS or BRAF gene mutations in colorectal cancer patients in Asia. Also included are studies on KRAS and BRAF gene mutations from fresh frozen, formalin-fixed paraffin-embedded (FFPE), or biopsied colorectal carcinoma specimens. Also, KRAS and BRAF studies involving more than one sample size and all related papers published at valid international summits were included. No limit is set on methods for determining gene mutations. The exclusion criteria include (1) studies not associated with frequency of KRAS and BRAF gene mutations, (2) studies that investigated just one of either codon 12 or codon 13 of KRAS gene mutation, (3) reviews and case reports, (4) KRAS and BRAF gene mutations that are related to cell lines and animal studies, and (5) studies that investigated BRAF gene mutation through KRAS-positive patients [16]. All authors were involved in the study screening, selection, and assessment criteria. Two authors (A.H.A. and A.A.I.) independently screened the articles based on title and abstract. This was proceeded by the assessment of the full texts. Any discord during the screening process were resolved by discussion with other authors in the study.

2.2. Data Extraction and Quality Assessment. The data extraction was carried out by using an Excel spreadsheet. Two reviewers (H.A.A. and A.A.I.) independently vetted the titles and abstracts and extracted crucial information required; study I.D, publication year, period and design, gene, data on the mutation of KRAS, and BRAF prevalence among patients diagnosed with colorectal cancer in Asia were diligently extracted. Any inconsistencies were handled through conversation with a third reviewer (S.M.S) to
prevent any sort of prejudice, and any discrepancies were sorted out via dialogue involving other reviewers to avoid any kind of bias. The quality of the methodological approach for the studies included was appraised independently by two authors (A.H.A. and Y.W.) via the Joanna Briggs Institute (JBI) critical appraisal checklist for prevalence data [17] (Supplementary JBI file). A score of 1 for “yes” and 0 for other parameters was allotted to obtain a sum quality score that ranges between 0 and 9. Studies with a final score of 7–9 were chosen to be of desirable quality. The studies within the latter acceptable score range were included in the data extraction phase of the meta-analysis.

2.3. Data Synthesis and Analysis. Data analysis was carried out via the use of OpenMeta Analyst and Comprehensive Meta-Analysis 3.0 (CMA 3.0) software [18]. The prevalence of KRAS and BRAF gene mutations among patients with colorectal carcinoma in Asia was calculated, and subgroup analysis was also carried out on location, tumour stage, tumour grade, and period of study. A random-effect model through the DerSimonian-Laird method of the meta-analysis was employed to obtain the pooled estimates of the reported KRAS and BRAF gene mutation cases. Further, to ascertain the study quality, possible publication bias was scrutinized by creating a funnel plot. The asymmetry of the plot was further investigated via Egger’s regression test [19]. The heterogeneities of study-level estimates were determined by Cochran’s Q test and quantified using $I^2$ statistics. $I^2$ values of 25%, 50%, and 75% were considered low, moderate, and high heterogeneities, respectively [20]. For all tests, a $P$ value < 0.001 was labelled statistically significant.

3. Result

This section is divided into subheadings to provide a concise and precise description of the experimental results and their interpretation, as well as the experimental conclusions that can be drawn from the outcomes.

3.1. Search Results and Study Selection. A total of 646 records were obtained by searching three electronic databases. After eliminating duplications and studies that do not favour the inclusion criteria, the remaining 498 were screened via titles and abstracts and by further excluding 261 records that satisfied the exclusion criteria and another 115 records that were done outside Asia; then, 122 records were left. Upon further scrutiny, another 34 records that did not merit the inclusion criteria as depicted in Figure 1 above were included in the data extraction phase of the meta-analysis.

![Figure 1: Summary of article identification and selection process.](image_url)
3.2. Characteristics of the Eligible Studies. The characteristics of studies on KRAS and BRAF mutations are illustrated in Tables 1 and 2, respectively. The meta-analysis study comprises of 25,525 sample sizes; all studies were from the Asian region with the male patient being most of the total participants, 15,743 out of 25,525 (61.7%). The comprehensive characteristics of the included studies are illustrated in Table 1.

3.3. Prevalence of KRAS Mutations in CRC Patients. The prevalence of KRAS gene mutation illustrated in the 59 studies included in the meta-analysis involves a total of 25,525 patients. Among the studies, the highest frequency of KRAS mutations reported by Rahadiani et al. [57] was 71.8% (95% CI: 55.9-83.6) (38), and the lowest frequency of KRAS mutations was reported by Bakarman and AlGarni [2] at a rate of 12.5% (95% CI: 9.1-17.0) (37). Using the random-effect model, the overall prevalence of KRAS mutations among Asians was 36.3% (95% CI: 34.5-38.2) with $I^2 = 85.54\%$ and $(P \text{ value} < 0.001)$ (Figure 2). Furthermore, in 43 out of 59 studies, the frequency of KRAS gene mutations was reported in codon 12 and codon 13. The prevalence of mutated codons across all KRAS mutations could be seen in supplementary figures SF1 and SF2. Codon 12 and codon 13 mutations were discovered in the populations to be 76.6% (95% CI: 74.2-78.8) and 21.0% (95% CI: 19.1-23.0), respectively (Supplementary figures SF1 and SF2).

3.4. Prevalence of KRAS Gene Mutation in Colorectal Cancer Stratified by Study Location and Period of Study. To determine the prevalence of KRAS mutation in CRC patients from various regions in Asia, a subgroup meta-analysis was undertaken. Data were available for nineteen locations from the listed studies, with the largest number of studies coming from China ($n$: 7) (Table 4; Supplementary Figure SF12).

The country of Indonesia had the highest prevalence rates projection at 71.8% (95% CI: 55.9–83.6), while Pakistan had the lowest estimate of 13% (95% CI: 8.8-19.8) (Table 3; Supplementary Figure SF3). Greater heterogeneity was found in studies from Saudi Arabia, China, South Korea, and India ($I^2 = 91.95\%, 91.21\%, 87.18\%$, and $83.77\%$), respectively ($P \text{ value} < 0.001$), which may have added to the overall heterogeneity found in the outcome.

On the period of study, studies done “after 2010” had the highest number of studies (28) during the study period (Table 3; Supplementary Figure SF4) with the highest KRAS prevalence at 39.9% (95% CI: 37.3–42.5), while those done “2010 and below” had KRAS prevalence at 32.3% (95% CI: 28.8–36.0), respectively ($P \text{ value} < 0.001$).

On the tumour stage, KRAS mutation was reported highest in the late stage at 67.9% (95% CI: 59.3–75.5), while on location, the colon recorded the highest KRAS mutation of 61.2% (95% CI: 55.1–67.0). On the grading of KRAS mutation in CRC, “Moderate grading” recorded the highest KRAS mutation of 51.8% (95% CI: 42.9–61.2) (Table 3; Supplementary Figure SF6, 8, and 10, respectively).

3.5. Prevalence of BRAF Gene Mutation of Patients with Colorectal Cancer Stratified by Forest Plot for BRAF. The prevalence of BRAF gene mutations in colorectal patients was investigated using the random-effect model. In the 29 (607 patients) out of 88 studies that reported BRAF prevalence, the highest prevalence was reported by Jauhri et al. [39] at 7.1% (95% CI: 3.6-13.6) and Yari et al. [67] at 7.0% (95% CI: 3.4-14.0), respectively. The least BRAF gene mutation was reported by Hsieh et al. [37] at 1.1% (95% CI: 0.3-4.3). In 2 out of the 29 studies, Kaji et al. [41] and Niya et al. [42] reported no BRAF mutation: 0% (95% CI: 0.0–7.6) and 0% (95% CI: 0.0–0.8), respectively. The overall prevalence of BRAF gene mutations was 5.6% (95% CI: 3.9-8.0) with $I^2 = 94.00\%$ and $(P \text{ value} < 0.001)$ (Figure 3). In all the studies (29 out of 88), the screening of BRAF gene mutations was based on the detection of BRAF-V600E mutation.

3.6. Subgroup Analysis of the Prevalence of BRAF Gene Mutation in Patients with Colorectal Cancer Stratified by Study Location and Period of Study Conduct. To determine the prevalence of BRAF in colorectal cancer CRC patients from various regions in Asia, a subgroup meta-analysis was undertaken. Data were available for fourteen locations from the listed studies, with the largest number of studies coming from China ($n$: 7) (Table 4; Supplementary Figure SF12).

India had the highest prevalence rate projection at 11.7% (95% CI: 6.2 – 21.0), while Taiwan had the lowest estimate of 1.1% (95% CI: 0.3-4.3) (Table 4; Supplementary Figure SF12). Greater heterogeneity was found in studies from China and Iran ($I^2 = 91.21\%$ and 96.16%), respectively ($P \text{ value} < 0.001$), which may have added to the overall heterogeneity found.

On the period of study, studies done “after 2010” had the highest number of studies (17) during the study period (Table 4; Supplementary Figure SF13) with the highest BRAF gene mutation prevalence at 5.4% (95% CI: 3.7–7.7), while those done “2010 and below” had BRAF mutation prevalence at 5.6% (95% CI: 2.0–14.6), respectively ($P \text{ value} < 0.001$).

On the tumour stage, BRAF mutation was reported highest in the “late stage” at 59.9% (95% CI: 48.2–70.7), while on location, “colon” recorded the highest BRAF mutation of 67.9% (95% CI: 37.3–42.5). On the grading of BRAF mutation in CRC, “moderate grading” recorded the highest BRAF mutation of 56.3% (95% CI: 43.3–68.6) (Table 4; Supplementary Figures SF15, 17, and 19).

3.7. Analyses of Sensitivity and Publication Bias. A funnel plot of random effects was generated to observe evidence of publication bias among the studies reporting KRAS gene mutation (Figure 4) and BRAF gene mutation (Figure 5) among Asian CRC patients. There was no clear evidence of publication bias in both KRAS and BRAF mutation studies.
Table 1: Major characteristics of the prevalence of KRAS screening studies that were included in the meta-analysis.

| S/ N | Author et al., Year | Location | Male, n (%) | Age (Mean ± SD) | Sample size | Tumour stage (early stage)* | Tumour stage (late stage)* | Tumour location (colon)* | Tumour location (rectum)* | Tumour grade (poorly differentiated)* | Tumour grade (moderately differentiated)* | Tumour grade (well-differentiated)* | Method | Total KRAS mutation (%) | KRAS (codon 12) % | KRAS (codon 13) % |
|------|---------------------|----------|-------------|-----------------|-------------|-----------------------------|----------------------------|--------------------------|------------------------------|----------------------------------------|----------------------------------------|----------------------------------------|---------|------------------------|-----------------|------------------|
| 1    | Al-Allawi et al., 2012 [21] | Iraq | 54 | 55.4 ± 15.25 | 50 | 50 | 50 | 56 | 18 | 62 | 20 | Sequeencing | 48 | 91.7 | 8.3 |
| 2    | Amirifard et al., 2016 [22] | Iran | 79 | 51.5 ± 12.6 | 33 | 0 | 100 | 55 | 45 | 9 | 21.2 | 69.8 | Sequeencing | 36.4 | 91.7 | 8.3 |
| 3    | Awidi et al., 2019 [23] | Jordan | 60 | 58 (19-83) | 190 | NR | NR | 97.4 | 2.63 | NR | NR | NR | Sequeencing | 48.4 | 81.5 | 17.4 |
| 4    | Bader and Ismail, 2014 [24] | Saudi Arabia | 58 | 55 (26-90) | 83 | 13.3 | 86.7 | 76 | 24 | 9.6 | 82 | 8.4 | Sequeencing | 42.2 | 88.6 | 11.4 |
| 5    | Bakarman and Al-Karni, 2019a [24] | Saudi Arabia | 56 | 57 ± 13 | 279 | 34 | 56.6 | 59.5 | 40.5 | 6.5 | 73.8 | 5.7 | Sequeencing | 12.5 | NR | NR |
| 6    | Bando et al., 2012 [25] | Japan | 60 | NR | 109 | NR | NR | 69.7 | 30.2 | NR | NR | NR | Sequeencing | 30.3 | 78.8 | 21.2 |
| 7    | Bishehsari et al., 2006 [26] | Iran | 57 | NR | 182 | NR | NR | 71 | 29 | NR | NR | NR | Sequeencing | 37.4 | 66.2 | 32.4 |
| 8    | Bagadi et al., 2012 [27] | India | 74 | 56 (23-93) | 100 | 22.5 | 77.5 | 78 | 22 | NR | NR | NR | Sequeencing | 23 | 87 | 13 |
| 9    | Chen et al., 2009 [28] | Taiwan | 54 | 25-90 | 90 | NR | NR | 68.9 | 31.1 | NR | NR | NR | Sequeencing | 35.6 | 75 | 25 |
| 10   | Dallol et al., 2016 [29] | Saudi Arabia | 59 | NR | 99 | NR | NR | 72.7 | 27.3 | 10.1 | 61.6 | 16.2 | HTT-sequencing | 35.4 | NR | NR |
| 11   | Deng et al., 2015 [30] | China | 59 | NR | 433 | 50.3 | 49.7 | 73.9 | 26.1 | 21.2 | 49.2 | 21.9 | Sequeencing | 38.3 | 74.1 | 25.9 |
| 12   | Dolkah et al., 2015 [31] | Iran | 77 | 77.6 (27-90) | 30 | 36.7 | 46.7 | NR | NR | 6.7 | 26.7 | 50 | Sequeencing | 20 | NR | NR |
| 13   | Dolkah et al., 2016 [32] | Iran | 65 | 61.9 ± 15.34 | 100 | 37 | 29 | 72 | 28 | 3 | 22 | 49 | Sequeencing | 26 | 61.5 | 34.6 |
| 14   | Elbjeirami and Sughayer, 2012 [33] | Jordan | 55 | 55 (22-74) | 100 | 5 | 95 | 78 | 22 | NR | NR | NR | Sequeencing | 44 | 88.6 | 11.4 |
| 15   | Elsamany et al., 2014 [34] | Saudi Arabia | 54 | NR | 116 | 23.3 | 76.7 | 67.8 | 32.2 | 29.3 | 58.7 | 12 | Sequeencing | 37.1 | NR | NR |
| 16   | Fu et al., 2019 [35] | China | 60 | 60 (14-96) | 5495 | NR | NR | 50.1 | 49.9 | 6.5 | 71.3 | 24.4 | HRMS | 37.7 | 75.1 | 22.2 |
| S/N | Author et al., Year | Location | Male, n (%) | Age | Sample size | Tumour stage (early stage)* | Tumour stage (late stage)* | Tumour location (colon)* | Tumour location (rectum)* | Tumour grade (poorly differentiated)* | Tumour grade (moderately differentiated)* | Tumour grade (well-differentiated)* | Method | Total KRAS mutation (%) | KRAS (codon 12) % | KRAS (codon 13) % |
|-----|---------------------|----------|-------------|-----|-------------|-----------------------------|-----------------------------|------------------------|------------------------|--------------------------------|--------------------------------|--------------------------------|---------|------------------------|----------------|----------------|
| 17  | He et al., 2020 [36] | China    | 62 59 (26-83) | 194 | 4.1         | 83                          | 72.6                        | 27.3                   | 45.9                   | NR                           | NR                           | NR                           | Sequencing | 42.3                   | 63.4           | 17.1           |
| 18  | Hsieh et al., 2012 [37] | Taiwan | NR NR        | 182 | NR NR       | NR                          | NR                          | NR NR                  | NR NR                  | NR                           | NR                           | NR                           | Sequencing | 33.5                   | NR            | NR             |
| 19  | Hamzezadeh et al., 2018 [38] | Iran    | 59 57 (27-86) | 87  | NR NR       | NR                          | 87.3                        | 12.6                   | 4.6                    | 77                           | 18.3                         | NR                           | Sequencing | 28.7                   | 72             | 28             |
| 20  | Jauhari et al., 2017a [39] | India   | 61 NR        | 112 | 23.2        | 76.8                        | 82.1                        | 17.9                   | NR NR                  | NR                           | NR                           | NR                           | Sequencing | 35.7                   | 67.5           | 17.5           |
| 21  | Jazi et al., 2017 [40] | Iran    | 56 61.2 ± 9.13 | 52 | 55.8        | 28.8                        | 28.8                        | 71.2                   | 15.4                   | 42.3                         | 23.1                         | NR                           | Sequencing | 15.4                   | 75             | 25             |
| 22  | Kaji et al., 2011 [41] | Japan   | 35 68.9 ± 9.8 | 98  | NR NR       | NR                          | NR                          | NR NR                  | NR NR                  | NR                           | NR                           | NR                           | NR                   | 60.2                   | 71.2           | 23.7           |
| 23  | Khorshideh Nia et al., 2016 [42] | Iran    | 57 NR        | 1000 | NR NR       | NR                          | NR                          | NR NR                  | NR NR                  | 16.4                         | 38.4                         | 43.9                         | HRMS      | 33.6                   | 85.1           | 14.9           |
| 24  | Korphaisarn et al., 2019 [14] | Thailand | 57 64 (30-89) | 108 | 24.1        | 75.9                        | 82.4                        | 17.6                   | 6.5                    | 86.1                         | 4.6                           | NR                           | NR                   | 47.2                   | NR            | NR             |
| 25  | Kwon et al., 2011 [43] | South Korea | 60 54 ± 12.33 | 92  | 0 100       | 56.5                        | 43.5                        | 15.2                   | 67.4                   | 12                           | 20.7                         | NR                           | NR                   | 20.7                   | NR            | NR             |
| 26  | Kaidanav et al., 2020 [44] | Kazakhstan | 45 56.4 + 10.5 | 332 | NR NR       | NR                          | NR                          | NR NR                  | NR NR                  | 16.4                         | 38.4                         | 43.9                         | HRMA/P    | 33.6                   | 85.1           | 14.9           |
| 27  | Koochak et al., 2016a [16] | Iran    | 57 NR        | 1000 | 0 100       | NR                          | NR                          | NR NR                  | 16.4                   | 38.4                         | 43.9                         | NR                           | HRMA/P    | 33.6                   | 85.1           | 14.9           |
| 28  | Kumar et al., 2015 [45] | Oman    | 59 56 (18-80) | 162 | 22.8        | 75.3                        | 29.6                        | 70.4                   | 16.7                   | 77.2                         | 5.6                           | IHC                        | NR                   | 23.5                   | NR            | NR             |
| 29  | Liao et al., 2014 [46] | Taiwan  | 54 63.2 (30-88) | 52 | 9.6        | 90.4                        | 67.3                        | 30.8                   | 5.8                    | 84.6                         | 3.8                           | PNA-M/PCR      | 28.8                   | 66.7           | 33.3           |
| 30  | Lee et al., 2020 [47] | South Korea | 43 62 (27-88) | 310 | 23.6        | 74.9                        | NR                          | NR NR                  | NR NR                  | 37.1                         | 76.5                         | 23.5                         | Sequencing | 37.1                   | 76.5           | 23.5           |
| 31  | Mohamed Suhaimi et al., 2015 [48] | Singapore | 54 58.5 (26-74) | 44  | 50         | 40.9                        | 54.5                        | 45.5                   | NR NR                  | NR                           | NR                           | NR                           | HRM-S     | 31.8                   | NR            | NR             |
| 32  | Mosen et al., 2016 [49] | Iran    | 70 62.17 ± 14.18 | 50 | NR NR       | NR                          | NR                          | 74 26                  | NR NR                  | NR                           | NR                           | NR                           | Sequencing | 28                     | 71.4           | 28.6           |
| 33  | Mulla et al., 2020 [50] | Saudi Arabia | 51 60 (28-91) | 51  | 35.3        | 64.7                        | NR                          | NR NR                  | 3.9                    | 84.3                         | 11.8                         | Histopathology | 39.2                   | 75             | 20             |
| 34  | Murtaza et al., 2014 [51] | Pakistan | 64 NR        | 150 | 12 88       | 48                          | 52                          | 38.7                   | 26.7                   | 34.7                         | Sequencing | 13.3                   | 60             | 35             |
| N  | Author et al., Year | Year | Location | Male, n (%) | Age | Sample size | Tumour stage (early stage) | Tumour stage (late stage) | Tumour location (colon) | Tumour location (rectum) | Tumour grade (poorly differentiated) | Tumour grade (moderately differentiated) | Tumour grade (well-differentiated) | Method                      | Total KRAS mutation ( % ) | KRAS (codon 12) % | KRAS (codon 13) % |
|----|---------------------|------|----------|-------------|-----|-------------|---------------------------|---------------------------|-------------------------|-------------------------|-------------------------------|------------------------------------------|-------------------------------|---------------------------|-----------------------------|---------------------|---------------------|---------------------|
| S/N | Author Year | Location | Male, n (%) | Age | Sample size | Tumour stage (early stage)* | Tumour stage (late stage)* | Tumour location (colon)* | Tumour location (rectum)* | Tumour grade (poorly differentiated)* | Tumour grade (moderately differentiated)* | Tumour grade (well-differentiated)* | Method | Total KRAS mutation (%) | KRAS (codon 12) % | KRAS (codon 13) % |
|-----|--------------|----------|-------------|-----|-------------|-----------------------------|-----------------------------|--------------------------|------------------------|---------------------------------|---------------------------------|---------------------------------|---------|------------------------|----------------|----------------|
| 53  | Zahrani et al., 2014 [70] | Saudi Arabia | 63 | 56.7 (21-88) | 150 | 16 | 84 | 78.7 | 21.3 | NR | NR | NR | Sequencing | 56 | 86.9 | 13 |
| 54  | Zekri et al., 2019 [71] | Saudi Arabia | NR | NR | 45 | NR | NR | NR | NR | NR | NR | NR | Sanger sequencing | 53.1 | NR | NR |
| 55  | Zekri et al., 2012 [72] | Saudi Arabia | 65 | 61 (21-80) | 46 | 30 | 70 | 83 | 17 | 15 | 83 | 2 | Sanger sequencing | 32.6 | 86.7 | 13 |
| 56  | Zhang et al., 2015 [73] | China | 59 | 62.1 (18-96) | 1110 | 19.1 | 80.9 | 50.7 | 49.3 | 7.5 | 73.5 | 19 | PCR-SS | 45.4 | 79 | 21 |
| 57  | Zhu et al., 2020 [74] | China | 70 | NR | 53 | 37.7 | 62.3 | NR | NR | NR | NR | NR | Sequencing | 47.2 | NR | NR |
| 58  | Zhang et al., 2018 [75] | China | 62 | 64 | 813 | 52.4 | 47.5 | 45.5 | 54.5 | 4.1 | 73.7 | 17 | Sequencing | 42.6 | NR | NR |
| 59  | Zihui Yong et al., 2020 [76] | Singapore | 53 | 62 (12-91) | 363 | 0 | 100 | 77 | 23 | NR | NR | NR | Sequencing | 34.7 | NR | NR |

N: number; NR: not reported. *Percentage of all samples, age is presented in years (mean ± SD/median (range/IQR)/range). HRMS: high resolution melting- (HRM-) sequencing; HRMA/P: high resolution melting assay/pyrosequencing; PNAM/PCR and PNAM/PCR/S: peptide nucleic acid-mediated polymerase chain reaction/sequencing; IHC: immunohistochemistry; SS: Sanger sequencing.
| Sr/ N | Author et al., Year | Country | Male, n (%) | Age | Sample size | Tumour stage (early stage)* | Tumour stage (late stage)* | Tumour location (colon)* | Tumour location (rectum)* | Tumour grade (poorly differentiated)* | Tumour grade (moderately differentiated)* | Tumour grade (well differentiated)* | Method | Total BRAF mutation (%) |
|-------|---------------------|---------|-------------|-----|-------------|-----------------------------|---------------------------|-------------------------|--------------------------|---------------------------------|---------------------------------|---------------------------------|---------|------------------------|
| 1     | Bagadi et al., 2012  | India   | 74 (23-93) | 56  | 100         | 22.5                        | 77.5                      | 78                      | 22                       | NR                              | NR                              | NR                              | Sequencing | 17                     |
| 2     | Eshkotizadeh et al., 2018  | India   | 49 (14-98) | 80  | 5495        | NR                          | NR                       | NR                      | NR                       | NR                              | NR                              | NR                              | Sequencing | 21.1                   |
| 3     | Fu et al., 2019 | China   | 60 (40-50) | 60  | NR          | NR                          | NR                       | 50.1                    | 0.499                    | 6.5                             | 0.713                           | 24.4                           | HRMS       | 2.8                    |
| 4     | Mohammadi Asl et al., 2014 | Iran    | 44.ynn (40-50) | 44.ynn (40-50) | 80 | NR          | NR                          | NR                      | NR                      | NR                              | NR                              | NR                              | PCR-FFLP/S | 46.3                   |
| 5     | He et al., 2020 | China   | 62 (26-83) | 59  | 194         | 4.1                         | 83                       | 72.6                    | 27.3                     | 45.9                            | NR                              | NR                              | Sequencing | 11.9                   |
| 6     | Hsieh et al., 2012   | Taiwan  | NR          | NR  | 182         | NR                          | NR                       | NR                      | NR                       | NR                              | NR                              | NR                              | Sequencing | 1.1                    |
| 7     | Isumi et al., 2017a   | India   | 61          | NR  | 112         | 23.2                        | 76.8                     | 82.1                    | 17.9                     | NR                              | NR                              | NR                              | Sequencing | 7.1                    |
| 8     | Kaji et al., 2011     | Japan   | 35          | NR  | 98          | 68.9 + 9.8                  | 45.1                    | 45.5                    | 38.4                     | 43.9                            | HRMS                             | 0                               | HRMS       | 0                      |
| 9     | Karbalal<strong>i</strong> Niya et al., 2016 | Iran    | 57          | NR  | 1000        | NR                          | NR                      | NR                      | NR                      | 16.4                            | 38.4                            | 43.9                            | HRMS       | 0                      |
| 10    | Korphaisarn et al., 2019 | Thailand | 57          | 64 (30-89) | 108 | 24.1          | 75.9                      | 82.4                    | 17.6                     | 6.5                             | 86.1                            | 4.6                             | PNAMPCR    | 1.9                    |
| 11    | Kwon et al., 2011     | South Korea | 60          | 54 ± 12.33 | 92  | 0            | 100                      | 56.5                    | 43.5                     | 15.2                            | 67.4                           | 12                              | PNAMPCR/S  | 3.3                    |
| 12    | Mohamed Suhaimi et al., 2015 | Singapore | 55          | 58.5 ± 26.74 | 44  | 50            | 40.9                     | 54.5                    | 45.5                     | NR                              | NR                              | NR                              | HRM-S      | 11.4                   |
| 13    | Nagabothu et al., 2019 | Japan    | NR          | NR  | 50          | NR                          | NR                      | NR                      | NR                       | NR                              | NR                              | NR                              | SS         | 6                      |
| 14    | Nguyen et al., 2021   | Vietnam | 56          | 59.94 ± 12.3 | 151 | 76.2          | 19.2                     | 68.9                    | 31.1                     | 10.2                            | 36.2                            | 53.2                            | SS         | 2.6                    |
| 15    | Rezak et al., 2010    | Israel  | 5           | NR  | 1297        | NR                          | NR                      | NR                      | NR                       | NR                              | NR                              | NR                              | Sequencing | 5                      |
| 16    | Saxena et al., 2018   | India   | 68          | 64 (26-90) | 65  | 60            | 40                      | 50.8                    | 26.2                     | Immunohistochemistry          | 4.6                             |                                  |            |                        |
| 17    | Shimada et al., 2018  | Japan   | 59          | NR  | 111         | NR                          | NR                      | NR                      | NR                       | NR                              | NR                              | NR                              | NGS        | 6.3                    |
| 18    | Siraj et al., 2014    | Saudi Arabia | 51          | NR  | 770         | 44.5                        | 49.2                    | NR                      | NR                       | 12.1                            | 76.6                            | 9.6                             | PCR/sequencing | 2.2                   |
| 19    | Song et al., 2020     | China   | 61          | NR  | 2356        | 44.8                        | 45.2                    | NR                      | NR                       | 18.7                            | NR                              | 81.3                            | Sequencing | 1                      |
| 20    | Taniguchi et al., 2020 | Japan    | NR          | 66  | 325         | NR                          | NR                      | NR                      | NR                       | NR                              | NR                              | NR                              | Sequencing | 10.5                   |
| 21    | Taniguchi et al., 2018 | Japan    | 59          | 64 (26-89) | 302 | 26.8          | 73.2                     | 53                      | 47                       | 9.6                             | NR                              | NR                              | Sequencing | 6                      |
| 22    | Vilkin et al., 2009   | Israel  | 47          | 67.6 ± 12.3 | 128 | 54.9          | 45.1                    | NR                      | NR                       | 25.1                            | 54.6                            | 20.3                            | Sequencing | 18.8                   |
| 23    | Wu et al., 2017       | China   | NR          | 1694 | NR          | NR                          | NR                      | NR                      | NR                       | NR                              | NR                              | NR                              | Sequencing | 4.2                    |
| Sr/ N | Author and Year | Location | Male, n (%) | Age | Sample size | Tumour stage (early stage)* | Tumour stage (late-stage)* | Tumour location (colon)* | Tumour location (rectum)* | Tumour grade (poorly differentiated)* | Tumour grade (moderately differentiated)* | Tumour grade (well-differentiated)* | Method | Total BRAF mutation (%) |
|-------|-----------------|----------|-------------|-----|-------------|---------------------------|--------------------------|------------------------|-------------------------------|---------------------------------|---------------------------------|--------------------------------|--------|------------------------|
| 24    | Wang et al., 2017 [85] | Indonesia | 46          | 56 ± 11.2 | 43 | NR | NR | 67.4 | 32.6 | 44.2 | 34.9 | 20.9 | Sequencing | 14 |
| 25    | Warsinggih et al., 2020 [86] | Indonesia | NR | NR | 100 | NR | NR | NR | NR | NR | NR | NR | Sequencing | 7 |
| 26    | Yip et al., 2020 [67] | Malaysia | 65 | NR | 44 | NR | NR | NR | NR | NR | NR | NR | Sequencing | 2.3 |
| 27    | Zhang et al., 2020 [87] | Malaysia | NR | 480 | 18.8 | NR | 81.3 | NR | NR | NR | NR | Sequencing | 4 |
| 28    | Zhang et al., 2015 [73] | China | NR | 1110 | 19.1 | NR | 80.9 | NR | NR | NR | NR | 19 | PCR-SS | 3.1 |
| 29    | Zhu et al., 2020 [74] | China | 70 | NR | 53 | NR | 37.7 | NR | 62.3 | NR | NR | NR | Sequencing | 11.3 |

N: number; NR: not reported. *Percentage of all samples, age is presented in years (mean ± SD/median (range/IQR)/range). HRMS: high resolution melting-(HRM-)sequencing; HRMA/P: high resolution melting assay/pyrosequencing; PNAM/PCR and PNAM/PCR/S: peptide nucleic acid-mediated polymerase chain reaction/sequencing; IHC: immunohistochemistry; SS: Sanger sequencing.
4. Discussion

Several research today showed that mutations in the RAS family of genes especially the KRAS are linked to around a third of all malignancies; however, the incidence of the gene mutations varies greatly depending on the kind of cancer: often seen to be 40% in colorectal cancer, 15-20% in non-small-cell lung cancer, and 95% in pancreas carcinoma [44]. Only a few individuals diagnosed with colorectal cancer would be opportune to receive curative surgery if detected early because, at the time of consult with the surgeon, it is already in the late stage wherein the prognosis is poor. More so, the illness involves no specific early presenting features, and the long-term disease period is usually associated with probable organ metastases [86, 88]. Also, because colorectal cancer is thought to grow progressively over time due to the buildup of genetic abnormalities, the threat of reoccurrence and mortality from colorectal cancer is significantly linked to the stage of the disease at diagnosis [86]. Although there is a tremendous advance in the CRC treatment via the use of newer chemotherapy and targeted therapies, the incidence of metastases remains a challenge for patients [86, 88].

Figure 2: A forest plot for the prevalence of KRAS mutation in Asian CRC patients.
of cytotoxic agents, i.e., monoclonal antibodies to targeted therapy such as on EGF receptor [78], CRC still poses a significant threat to life as KRAS gene mutation is reported as a major cause of treatment failure in cancer therapy [89].

Colorectal cancer (CRC) is the third most frequent cancer in the world, with 2.0 million new cases in 2020, accounting for 11% of all new cancer cases [90]. It was estimated as 1.9 million of all new cases and 880,000 deaths in 2018 [91]. The incidence and mortality rates of colorectal cancer (CRC) differ significantly around the globe, i.e., differs in various regions. From a total of 646 eligible papers that were filtered in this study, 88 studies were finally selected to investigate the prevalence of KRAS and BRAF gene mutations in this analysis. During this analysis, approximately 115 articles reporting KRAS and BRAF gene mutations in CRC outside Asia were identified, but they were, however, excluded because they did not fulfill the study’s inclusion criteria. This plethora of articles discovered spanned almost every corner of the world. Balschun et al. [92] documented the prevalence case of KRAS and BRAF in German patients in Europe. Di Fiore et al. [93] reported the first instance of KRAS and BRAF mutations in CRC in the United Kingdom. Raskin et al. [94] and Osasan [95] studies were done in Africa. Altogether, these illustrated the different prevalence of KRAS mutation existence in CRC around the globe.

In this study, the prevalence of KRAS and BRAF mutations was investigated in 88 studies involving 25,527 CRC patients. The prevalence of KRAS and BRAF gene mutations was investigated in 88 studies involving 25,527 CRC patients. The prevalence of KRAS and BRAF gene mutations was investigated in 88 studies involving 25,527 CRC patients.

Table 3: Subgroup analysis. Prevalence of KRAS of patients with colorectal cancer stratified by study location of study.

| Location       | No. of Studies | Prevalence (%) | 95% CI       | I² (%) | Q    | Heterogeneity test |
|----------------|----------------|----------------|--------------|--------|------|--------------------|
|                |                |                |              |        |      |                    |
| Iraq           | 1              | 48.0           | 0.346-0.617  | NA     | NA   | NA                 |
| Iran           | 12             | 32.2           | 0.293-0.353  | 45.35  | 20.129 | 11 0.044           |
| Jordan         | 2              | 46.9           | 0.412-0.527  | 0.514  | 1    | 0.474              |
| Saudi Arabia   | 9              | 35.7           | 0.265-0.460  | 91.95  | 99.408 | 8 0.001            |
| Japan          | 8              | 40.1           | 0.355-0.448  | 77.04  | 30.494 | 7 0.001            |
| India          | 3              | 34.0           | 0.237-0.461  | 83.77  | 12.320 | 2 0.002            |
| Taiwan         | 3              | 33.4           | 0.284-0.387  | 0      | 0.672  | 2 0.715            |
| China          | 7              | 39.9           | 0.361-0.439  | 91.21  | 68.226 | 6 0.001            |
| Thailand       | 1              | 47.2           | 0.380-0.566  | NA     | NA   | NA                 |
| South Korea    | 3              | 34.3           | 0.246-0.456  | 87.18  | 15.600 | 2 0.001            |
| Kazakhstan     | 1              | 44.9           | 0.396-0.503  | NA     | NA   | NA                 |
| Oman           | 1              | 23.5           | 0.176-0.306  | NA     | NA   | NA                 |
| Singapore      | 2              | 34.4           | 0.299-0.392  | 0      | 0.145  | 1 0.703            |
| Pakistan       | 1              | 13.3           | 0.088-0.198  | NA     | NA   | NA                 |
| Vietnam        | 1              | 37.1           | 0.298-0.451  | NA     | NA   | NA                 |
| Indonesia      | 1              | 71.8           | 0.559-0.836  | NA     | NA   | NA                 |
| Sri Lanka      | 1              | 23.1           | 0.161-0.320  | NA     | NA   | NA                 |
| Israel         | 1              | 44.9           | 0.402-0.497  | NA     | NA   | NA                 |
| Malaysia       | 1              | 25.0           | 0.144-0.397  | NA     | NA   | NA                 |
| Overall        | 59             | 36.3           | 0.345-0.382  | 85.54  | 401.015 | 58 0.001           |

KRAS subgroup by period of study conduct

|               | No. of Studies | Prevalence (%) | 95% CI       | I² (%) | Q    | Heterogeneity test |
|---------------|----------------|----------------|--------------|--------|------|--------------------|
| 2010 and below| 19             | 32.3           | 0.288-0.360  | 90.78  | 187.902 | 18 0.001           |
| After 2010     | 28             | 39.9           | 0.373-0.425  | 82.25  | 152.081 | 27 0.001           |
| Early tumour stage¹ | 27  | 30.3           | 0.224-0.395  | 96.11  | 768.164 | 26 0.001           |
| Late tumour stage² | 27  | 67.9           | 0.593-0.755  | 82.25  | 668.459 | 26 0.001           |

KRAS subgroup by tumour location

|               | No. of Studies | Prevalence (%) | 95% CI       | I² (%) | Q    | Heterogeneity test |
|---------------|----------------|----------------|--------------|--------|------|--------------------|
| Colon         | 26             | 61.2           | 0.551-0.670  | 92.78  | 346.249 | 25 0.001           |
| Rectum        | 26             | 39.3           | 0.336-0.453  | 92.34  | 326.498 | 25 0.001           |

KRAS subgroup by tumour grading

|               | No. of Studies | Prevalence (%) | 95% CI       | I² (%) | Q    | Heterogeneity test |
|---------------|----------------|----------------|--------------|--------|------|--------------------|
| Poor          | 23             | 9.6            | 0.063-0.145  | 90.420 | 229.651 | 22 0.001           |
| Moderate      | 23             | 52.1           | 0.429-0.612  | 94.777 | 421.176 | 22 0.001           |
| Well          | 23             | 31.0           | 0.214-0.425  | 96.266 | 589.219 | 22 0.001           |

¹Implies stages 1 and 2; ²implies stages 3 and 4.
patients from various countries in Asia; the overall prevalence of KRAS gene mutations was found to be 36.3% (95% CI: 34.5-38.2). KRAS gene mutations are a well-investigated mutation in several carcinomas such as melanoma [96], non-small-cell lung carcinoma [97], colorectal carcinoma [98, 99], and papillary thyroid cancer [100]. KRAS gene mutations, which function as an active oncogene, are found in 35 to 45 percent of CRC cases globally [95% CI: 34.5-38.2], than when compared to those screened before 2010, 36.7% (95% CI: 34.6-38.8), than when compared to those screened before 2010, 36.3% (95% CI: 34.6-38.8), and probably due to medical advances and more medical screening [126]. During the shedding of tumour cells or apoptosis, small DNA fragments flow into the blood system, leading to the detection of this cDNA mutation in almost all cancer types and in the late stages of the tumour or the malignancy, hence, more frequent of the DNA mutation detection on screening.

The dynamic of gene expression patterns on gender and age was investigated by some studies as a possible risk for developing CRC [120, 121]. In this study, the age of the participants was also taken into consideration, the bulk of the recruited participants were adults, with most of them being over 50 years old, implying that KRAS gene mutation predominates in adult CRC. It was indeed as anticipated, given that older age has hitherto been identified as a health risk for CRC in numerous investigations [122]. Although data on gender were not reported for some studies in the included studies for this analysis, CRC was found to be more common in male patients (60.7%) than female patients (39.3%). This information points to the importance of gender predilection in the occurrence of CRC which is consistent with findings from other studies around the world [123, 124]. On the location of the tumour, the cancer was mostly found in the colon (82.1%) which is a similar finding in several studies [39], probably because the patient would present at the latter stage of cancer [125].

Although human scientific knowledge has greatly advanced compared to decades ago, however, our study found that the prevalence of KRAS gene mutation was higher among patients screened “after 2010,” 36.7% (95% CI: 34.6-38.8), than when compared to those screened “before 2010,” 32.3% (95% CI: 28.8-36.0), probably due to medical advances and more medical screening [126]. During the shedding of tumour cells or apoptosis, small DNA fragments flow into the blood system, leading to the detection of this cDNA mutation in almost all cancer types and in the late stages of the tumour or the malignancy, hence, more frequency of the DNA mutation detection on screening. Another reason could range from lifestyle evolution to dietary choice, synergically working together to modify our body biocomposition and genetic make-up [127]. The late-stage (stages 3 and 4) recorded more KRAS gene mutation (68%) than the early stage (30%) but this could be associated with discrepancies in the time of consultation and stages of cancer.
the tumour at the time of recruitment of the patients for the included studies, as the majority of the mean age reported by the studies was in 5th or 6th decade of life and because most of the patient would have distant metastases at the period of diagnosis.

On the location of KRAS and BRAF gene mutations, the colon (61% and 68%), respectively, was the most recorded mutation site which on the contrary is the rectum [128]; however, this is as expected as the main physiological function of the intestinal lumen of the colon includes water absorption and stool storage. Therefore, the contents contained inside the colon are relatively desiccated which is the tumour-conducive condition for gene mutation detection [129, 130]. In this present research, the majority of KRAS mutations occurred in codon 12, 76.6% (95% CI: 74.2-78.8), than in codon 13, 21.0% (95% CI: 19.1-23.0). These findings are comparable to those of previous research [105, 116, 117]. For example, in a Belgian research, 36.3 percent of people had KRAS mutations, with 91 percent of mutations in codon 12 [131]. Another study published in Dobre et al. [132] found that KRAS mutations in codons 12 and 13 were found in 79.3 percent and 19.7 percent of people, respectively. A similar study in Brazil reported that 87% of KRAS mutations were in codon 12 and 13% in codon 13. However, research in the Greek population found that KRAS mutations at codon 12 are uncommon (29.3%) [133]. Only 3 studies of the colorectal cancer patients in our analysis had a KRAS codon 61 mutations [26, 67, 68] which is not surprising given that the majority of KRAS mutations reported in human tumours are in codon 12, with mutations in codons 13 and 61 accounting for only 1.7-9 percent [27].

BRAF is also a member of the RAF gene subfamily that, like KRAS, performs its function in the EGFR downstream cascade, but their mutations are less frequent than the KRAS gene mutations. Among the BRAF gene, BRAFV600E mutation is the most prevailing [134], and in this present study, BRAFV600E mutation is used to examine the prevalence of BRAF gene mutation in CRC. The frequency of BRAF mutation varies globally, approximately 1.1–25% [16, 49, 135–139]. The prevalence of BRAF mutation obtained in this study was 5.6% (95% CI: 3.9-8.0), and this is conforming

| Subgroup        | No. of Studies | Prevalence (%) | 95% CI      | $I^2$ (%) | Q       | Heterogeneity test | Heterogeneity test DF | Heterogeneity test P |
|-----------------|----------------|----------------|-------------|-----------|---------|-------------------|------------------------|-----------------------|
| India           | 4              | 11.7           | 0.062-0.210 | 73.69     | 11.401  | 3                 | 0.010                  |                       |
| China           | 7              | 4.0            | 0.025-0.063 | 93.42     | 91.205  | 6                 | 0.001                  |                       |
| Iran            | 3              | 4.7            | 0.004-0.403 | 96.16     | 52.129  | 2                 | 0.001                  |                       |
| Taiwan          | 1              | 1.1            | 0.003-0.043 | NA        | NA      | NA                | NA                     |                       |
| Japan           | 5              | 6.9            | 0.044-0.107 | 55.86     | 9.062   | 4                 | 0.060                  |                       |
| Thailand        | 1              | 1.9            | 0.005-0.071 | NA        | NA      | NA                | NA                     |                       |
| South Korea     | 1              | 3.3            | 0.011-0.096 | NA        | NA      | NA                | NA                     |                       |
| Singapore       | 1              | 11.4           | 0.048-0.245 | NA        | NA      | NA                | NA                     |                       |
| Vietnam         | 1              | 2.6            | 0.010-0.068 | NA        | NA      | NA                | NA                     |                       |
| Israel          | 2              | 9.8            | 0.025-0.316 | 96.90     | 32.271  | 1                 | 0.001                  |                       |
| Saudi Arabia    | 1              | 2.2            | 0.014-0.035 | NA        | NA      | NA                | NA                     |                       |
| Indonesia       | 1              | 14.0           | 0.064-0.278 | NA        | NA      | NA                | NA                     |                       |
| Malaysia        | 1              | 2.3            | 0.003-0.144 | NA        | NA      | NA                | NA                     |                       |
| Overall         | 29             | 5.6            | 0.039-0.080 | 94.00     | 466.942 | 28                | 0.001                  |                       |
| BRAF subgroup by period of study conduct | | | | | | |
| 2010 and below  | 8              | 5.6            | 0.020-0.146 | 96.45     | 196.928 | 7                 | 0.001                  |                       |
| After 2010      | 17             | 5.4            | 0.037-0.077 | 91.27     | 183.302 | 16                | 0.001                  |                       |
| BRAF subgroup by tumour stage | | | | | | |
| Early tumour stage$^1$ | 10             | 40.1           | 0.293-0.518 | 62.297    | 62.297  | 9                 | 0.005                  |                       |
| Late tumour stage$^2$ | 10             | 59.9           | 0.482-0.707 | 95.59     | 62.297  | 9                 | 0.005                  |                       |
| BRAF subgroup by tumour location | | | | | | |
| Colon           | 10             | 67.9           | 0.577-0.766 | 54.421    | 19.746  | 9                 | 0.020                  |                       |
| Rectum          | 10             | 32.1           | 0.234-0.423 | 54.421    | 19.746  | 9                 | 0.020                  |                       |
| BRAF subgroup by tumour grade | | | | | | |
| Poor            | 11             | 30.4           | 0.189-0.450 | 88.066    | 83.794  | 10                | 0.001                  |                       |
| Moderate        | 11             | 56.3           | 0.432-0.686 | 86.413    | 73.599  | 10                | 0.001                  |                       |
| Well            | 11             | 10.2           | 0.056-0.179 | 69.996    | 33.329  | 10                | 0.001                  |                       |

$^1$Implies stages 1 and 2; $^2$Implies stages 3 and 4.
with the several existing findings, i.e., 1.1 to 5.8% in Asian studies and 5–21% in western studies [112, 131, 134, 140–142]. Another reason for these prevalence similarities could be associated with genetic homogeneity as the studies involve certain regions, and their lifestyles and diet are almost similar [143]. BRAF-activating mutations are frequently exclusive with KRAS mutations, accounting for 5–15% of mCRC cases, and are linked to a poor prognosis in stages II, III, and IV [144]. This mutation causes a constant stimulation of the mitogen-activating protein kinase MAPK pathway, which controls the transcriptase activity of regulatory genes in the cell cycle by modulating cell growth stimuli, a nonfunctioning condition that predisposes to cancerous growth [145].

This study possesses several merits and strengths. First, to the best of the author’s knowledge, it has been the first systematic review and meta-analysis carried out on the prevalence of KRAS and BRAF mutations among Asians with CRC. Also, a well-detailed and comprehensive search strategy ensures that elaborate all-inclusive papers are included, thus leading to a very large population size of 25,525. This also ensures high confidence in the outcomes obtained since
the included studies were of high methodology quality. However, this analysis was not without some limitations, with many linked to the data from the literature of the included studies such as small sample size, incomplete reports on sex, mean age, period of study conduction, differentiation, and location of the tumour, and lastly, mutation screening was done just for the B BRAFV600E. All these parameters/characteristics that would be crucial in upholding the study appraisal were not reported in some of the studies analysed in this meta-analysis, thus accounting to some of the heterogeneity seen in the studies.

5. Conclusions
This systematic review and meta-analysis study, which to the best of our knowledge, is the first to report on the prevalence of KRAS (36.6%) and BRAF (5.6%) mutations in CRC patients in Asia. The result showed that the rate of KRAS and BRAF gene mutations in CRC among Asians is rising. The adult age was more associated with CRC prevalence, and the males have increase fold and poorer outcome than their female counterparts. Despite some limitations, the meta-analysis yielded impressive results. The total prevalence of KRAS and BRAF mutations, 36.6% and 5.6%, respectively, differs in various countries in Asia according to this meta-analysis. Furthermore, when the findings of this study were compared to those of other studies, it was discovered that the prevalence of these mutations obtained in our analysis conformed with them.

Data Availability
All data accessed and analysed in this study are available in the article and its Supplementary Materials.

Disclosure
The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Conflicts of Interest
The authors declare no conflict of interest.

Acknowledgments
The authors would like to give appreciation to the Universiti Sains Malaysia, for availing us the support to undergo this work. This project was supported by the USM Fellowship Scheme.

Supplementary Materials
Supplementary Figure File SF1-20: search strategy, forest plot of the pooled prevalence of KRAS and BRAF in colorectal cancer CRC patients in Asia stratified by study location, period of study, tumour location, tumour stage, and tumour grade; JBI file: Joanna Briggs Institute (JBI) critical appraisal checklist for prevalence studies; PRISMA file: quality assessment of included studies. (Supplementary Materials)

References
[1] H. Sung, J. Ferlay, R. L. Siegel et al., “Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries,” CA: a Cancer Journal for Clinicians, vol. 71, no. 3, p. 209, 2021.
[2] M. A. Bakarman and A. M. AlGarni, “Colorectal cancer patients in western Saudi Arabia,” Saudi Medical Journal, vol. 40, no. 12, pp. 1227–1234, 2019.
[3] F. Bray, J. Ferlay, I. Soerjomataram, R. L. Siegel, L. A. Torre, and A. Jemal, “Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries,” CA: a Cancer Journal for Clinicians, vol. 68, no. 6, pp. 394–424, 2018.
[4] Y. Li, X. He, Y. E. Ding, H. Chen, and L. Sun, “Statin uses and mortality in colorectal cancer patients: an updated systematic review and meta-analysis,” Cancer Medicine, vol. 8, no. 6, pp. 3305–3313, 2019.
[5] M. C. Wong, H. Ding, J. Wang, P. S. F. Chan, and J. Huang, “Prevalence and risk factors of colorectal cancer in Asia,” Intestinal Research, vol. 17, no. 3, pp. 317–329, 2019.
[6] PRO, NTAP, Colorectal Cancer – Clinical Trials Landscape, Asia Pacific, 2021.
[7] M. Jauhri, A. Bhatnagar, S. Gupta et al., “Prevalence and coexistence of KRAS, BRAF, PIK3CA, NRAS, TP53, and APC mutations in Indian colorectal cancer patients: next-generation sequencing-a based cohort study,” Tumor Biology, vol. 39, no. 2, 2017.
[8] C. L. Nigro, V. Ricci, D. Vivenza et al., “Prognostic and predictive biomarkers in metastatic colorectal cancer anti-EGFR therapy,” World Journal of Gastroenterology, vol. 22, no. 30, p. 6944, 2016.
[9] A. Alharbi, H. Bin Dokhi, G. Almuhaini, F. Alomran, E. Masuadi, and N. Alomran, “Prevalence of colorectal cancer biomarkers and their impact on clinical outcomes in Riyadh,” Plos One, vol. 16, no. 5, article e0249590, 2021.
[10] S. Sayhan and D. S. Kahraman, “Pathologic features of colorectal carcinomas,” in Colon Polyps and Colorectal Cancer, pp. 455–480, Springer, 2021.
[11] J. A. McCubrey, L. S. Steelman, S. L. Abrams et al., “Roles of the RAF/MEK/ERK and PI3K/Pten/AKT pathways in malignant transformation and drug resistance,” Advances in Enzyme Regulation, vol. 46, no. 1, pp. 249–279, 2006.
[12] W. H. Gmeiner, “Recent advances in our knowledge of mCRC tumor biology and genetics: A focus on targeted therapy development,” OncoTargets and Therapy, vol. 14, p. 2121, 2021.
[13] T. J. Price, J. E. Hardingham, C. K. Lee et al., “Impact of KRAS and BRAF gene mutation status on outcomes from the phase III AGITG MAX Trial of capecitabine alone or in combination with bevacizumab and mitomycin in advanced colorectal cancer,” Journal of Clinical Oncology, vol. 29, no. 19, pp. 2675–2682, 2011.
[14] K. Korphaisarn, A. Pongpaibul, E. Roothummong et al., “High frequency of KRAS codon 146 and FBXW7 mutations in Thai patients with stage II-III colon cancer,” Asian Pacific Journal of Cancer Prevention, vol. 20, no. 8, pp. 2319–2326, 2019.
[15] D. Moher, A. Liberati, J. Tetzlaff, D. G. Altman, and PRISMA Group, “Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement,” Annals of Internal Medicine, vol. 6, no. 7, article e1000097, pp. 264–269, 2009.
[16] N. Rakhshani, M. H. K. Niya, F. S. Tameshkel et al., “Mutation analysis of KRAS and BRAF genes in metastatic colorectal cancer: a first large scale study from Iran,” Asian Pacific Journal of Cancer Prevention, vol. 17, no. 2, pp. 603–608, 2016.

[17] Z. Munn, S. Moola, K. Lisy, D. Riitano, and C. Tufanaru, “Methodological guidance for systematic reviews of observational epidemiological studies reporting prevalence and cumulative incidence data,” International Journal of Evidence-Based Healthcare, vol. 13, no. 3, pp. 147–153, 2015.

[18] W. Viechtbauer, “Conducting meta-analyses in R with the metafor package,” Journal of Statistical Software, vol. 36, no. 3, pp. 1–48, 2010.

[19] M. Egger, G. D. Smith, M. Schneider, and C. Minder, “Bias in meta-analysis detected by a simple,” Bmj, vol. 315, no. 7109, pp. 629–634, 1997.

[20] J. P. Higgins and S. G. Thompson, “Quantifying heterogeneity in a meta-analysis,” Statistics in Medicine, vol. 21, no. 11, pp. 1539–1558, 2002.

[21] N. A. Al-Allawi, A. T. Ismaeel, N. Y. Ahmed, and N. S. Merza, “The frequency and spectrum of K-ras mutations among Iraqi patients with sporadic colorectal carcinoma,” Indian Journal of Cancer, vol. 49, no. 1, pp. 163–168, 2012.

[22] N. Amirifard, E. Sadeghi, N. Farshchian, A. Haghparast, and M. Choubsaz, “Evaluation of KRAS gene mutations in metastatic colorectal cancer patients in Kermanshah province,” Asian Pacific Journal of Cancer Prevention, vol. 17, no. 7, pp. 3085–3088, 2016.

[23] M. Awidi, N. Ababneh, M. Shomaf et al., “KRAS and N Ras mutational gene profile of metastatic colorectal cancer patients in Jordan,” PLoS One, vol. 14, no. 12, 2019.

[24] T. Bader and A. Ismail, “Higher prevalence of KRAS mutations in colorectal cancer in Saudi Arabia: propensity for lung metastasis,” Alexandria Journal of Medicine, vol. 50, no. 3, pp. 203–209, 2014.

[25] H. Bando, T. Yoshino, S. Yuki et al., “Clinical outcome of Japanese metastatic colorectal cancer patients harbouring the KRAS p.G13D mutation treated with cetuximab + irinotecan,” Japanese Journal of Clinical Oncology, vol. 42, no. 12, pp. 1146–1151, 2012.

[26] F. Bishhehsari, M. Mahdavinia, R. Malekzadeh et al., “Patterns of K-ras mutation in colorectal carcinomas from Iran and Italy (a Gruppo Oncologico dell’Italia Meridionale study): influence of microsatellite instability status and country of origin,” Annals of Oncology, vol. 17, Supplement_7, p. vi91, 2006.

[27] S. B. Bagadi, M. Sanghvi, S. B. Nair, and B. R. Das, “Combined mutational analysis of KRAS, NRAS and BRAF genes in Indian patients with colorectal carcinoma,” International Journal of Biological Markers, vol. 27, no. 1, pp. 27–33, 2012.

[28] Y.-L. Chen, Y. S. Chang, J. G. Chang, and S. M. Wu, “Genotyping of K-ras codons 12 and 13 mutations in colorectal cancer by capillary electrophoresis,” Journal of Chromatography A, vol. 1216, no. 26, pp. 5147–5154, 2009.

[29] A. Dallol, A. Buhmeida, M. S. al-Ahwal et al., “Clinical significance of frequent somatic mutations detected by high-throughput targeted sequencing in archived colorectal cancer samples,” Journal of Translational Medicine, vol. 14, no. 1, p. 118, 2016.

[30] Y. Deng, L. Wang, S. Tan et al., “KRAS as a predictor of poor prognosis and benefit from postoperative FOLFOX chemotherapy in patients with stage II and III colorectal cancer,” Molecular Oncology, vol. 9, no. 7, pp. 1341–1347, 2015.

[31] R. Dolatkah, S. Dastgiri, M. H. Somi et al., “Common KRAS and BRAF mutations in colorectal cancer patients,” Govaresh, vol. 20, no. 1, pp. 27–33, 2015.

[32] R. Dolatkah, M. H. Somi, I. Asvadi Kermani et al., “Association between proto-oncogene mutations and clinicopathologic characteristics and overall survival in colorectal cancer in East Azerbaijan, Iran,” OncoTargets and Therapy, vol. 9, pp. 7385–7395, 2016.

[33] W. M. Elbjeirami and M. A. Sughayer, “KRAS mutations and subtyping in colorectal cancer in Jordanian patients,” Oncology Letters, vol. 4, no. 4, pp. 705–710, 2012.

[34] S. A. Elsamy, A. S. Alzahrani, M. M. Mohamed et al., “Clinico-pathological patterns, and survival outcome of colorectal cancer in young patients: western Saudi Arabia experience,” Asian Pacific Journal of Cancer Prevention, vol. 15, no. 13, pp. 5239–5243, 2014.

[35] X. Fu, Y. Huang, X. Fan et al., “Demographic trends and KRAS/BRAFV600E mutations in colorectal cancer patients of South China: a single-site report,” International Journal of Cancer, vol. 144, no. 9, pp. 2109–2117, 2019.

[36] K. He, Y. Wang, Y. Zhong, X. Pan, L. Si, and J. Lu, “Kras codon 12 mutation is associated with more aggressive invasiveness in synchronous metastatic colorectal cancer (mCRC): retrospective research,” Oncotargets and Therapy, vol. 13, pp. 12601–12613, 2020.

[37] L.-J. Hsieh, T. K. Er, C. C. Chen, J. S. Hsieh, J. G. Chang, and T. C. Liu, “Characteristics and prevalence of KRAS, BRAF, and PIK3CA mutations in colorectal cancer by high-resolution melting analysis in a Taiwanese population,” Clinica Chimica Acta, vol. 413, no. 19-20, pp. 1605–1611, 2012.

[38] L. Hamzehzadeh, F. Khadangi, E. Ghayoor Karimian, A. Pasdar, and M. A. Kerachian, “Common KRAS and NRAS gene mutations in sporadic colorectal cancer in Northeastern Iranian patients,” Current Problems in Cancer, vol. 42, no. 6, pp. 572–581, 2018.

[39] M. Jauhari, A. Bhatnagar, S. Gupta et al., “Prevalence and coexistence of KRAS, BRAF, PIK3CA, NRAS, TP53, and APC mutations in Indian colorectal cancer patients: next-generation sequencing-based cohort study,” Tumour Biology, vol. 39, no. 2, 2017.

[40] M. S. Jazi, S. Zahiri, S. L. Navid, and A. Talebi, “Relationship between common KRAS gene mutations and clinicopathological features of patients with colorectal cancer in Isfahan, Iran,” Govaresh, vol. 22, no. 1, pp. 39–46, 2017.

[41] E. Kaji, J. Kato, H. Suzuki et al., “Analysis of K-ras, BRAF, and PIK3CA mutations in laterally-spreading tumors of the colorectum,” Journal of Gastroenterology and Hepatology, vol. 26, no. 3, pp. 599–607, 2011.

[42] M. H. K. Niya, A. Basi, A. Koochak et al., “Sensitive high-resolution melting analysis for screening of KRAS and BRAF mutations in Iranian human metastatic colorectal cancers,” Asian Pacific Journal of Cancer Prevention, vol. 17, no. 12, pp. 5147–5152, 2016.

[43] M. J. Kwon, S. E. Lee, S. Y. Kang, and Y. L. Choi, “Frequency of KRAS, BRAF, and PIK3CA mutations in advanced colorectal cancers: comparison of peptide nucleic acid-mediated PCR clamping and direct sequencing in formalin-fixed, paraffin-embedded tissue,” Pathology - Research and Practice, vol. 207, no. 12, pp. 762–768, 2011.
[44] D. R. Kaydarova, K. K. Smagulova, N. A. Chichua, E. A. Ukolova, A. Z. Kurmankulova, and E. I. Ishkinin, “Study of the relationship between Kras gene mutations and gender, age and race in colorectal cancer patients residing in the Republic of Kazakhstan,” Siberian Journal of Oncology, vol. 19, no. 1, pp. 82–89, 2020.

[45] S. Kumar, I. A. Burney, K. F. Zahid et al., “Colorectal cancer patient characteristics, treatment and survival in Oman - a single center study,” Asian Pacific Journal of Cancer Prevention, vol. 16, no. 12, pp. 4853–4858, 2015.

[46] Y.-B. Kuo, J. S. Chen, C. W. Fan, Y. S. Li, and E. C. Chan, “Comparison of KRAS mutation analysis of primary tumors and matched circulating cell-free DNA in plasmas of patients with colorectal cancer,” Clinica Chimica Acta, vol. 433, pp. 284–289, 2014.

[47] H. S. Lee, D. Y. Hwang, and H. S. Han, “Histology and its prognostic effect on KRAS-mutated colorectal carcinomas in Korea,” Oncology Letters, vol. 20, no. 1, pp. 655–666, 2020.

[48] N.-A. Mohamed Suhaimi, Y. M. Foong, D. Y. S. Lee et al., “Non-invasive sensitive detection of KRAS and BRAF mutation in circulating tumor cells of colorectal cancer patients,” Molecular Oncology, vol. 9, no. 4, pp. 850–860, 2015.

[49] M. Naseri, A. Sebzari, F. Haghighi, F. Hajiipoor, and F. Emadian Razavi, “Frequency of K-RAS and N-RAS gene mutations in colorectal cancers in southeastern Iran,” Asian Pacific Journal of Cancer Prevention, vol. 17, no. 9, pp. 4511–4515, 2016.

[50] N. Mulla, A. Alshareef, A. R. Syed, and M. al-Jahel, “Clinicopathological study of K-ras mutations in colorectal tumors: a single-center retrospective study of 51 patients in Madinah, Saudi Arabia,” Careus, vol. 12, no. 8, article e9978, 2020.

[51] B. N. Murtaza, A. Bibi, M. U. Rashid, Y. I. Khan, M. S. Chaudri, and A. R. Shakoori, “Spectrum of K ras mutations in Pakistani colorectal cancer patients,” Brazilian Journal of Medical and Biological Research, vol. 47, no. 1, pp. 35–41, 2014.

[52] Y. Nagakubo, Y. Hirotsu, K. Amemiya, T. Oyama, H. Mochizuki, and M. Omata, “Accurate detection of KRAS, NRAS and BRAF mutations in metastatic colorectal cancers by bridged nucleic acid-clamp real-time PCR,” BMC Medical Genomics, vol. 12, no. 1, 2019.

[53] H. T. Nguyen, D. T. Le, Q. H. Duong, V. B. Tatipamula, and B. V. Nguyen, “High frequency of microsatellite instability and its substantial co-existence with KRAS and BRAF mutations in Vietnamese patients with colorectal cancer,” Oncology Letters, vol. 21, no. 1, 2020.

[54] N. Omidifar, B. Geramizadeh, and M. Mirzai, “K-ras mutation in colorectal cancer, a report from Southern Iran,” Iranian Journal of Medical Sciences, vol. 40, no. 5, pp. 454–460, 2015.

[55] S. M. Park, S. B. Choi, Y. S. Lee, and I. K. Lee, “Predictive value of KRAS mutation and excision repair cross-complementing 1 (ERCC1) protein overexpression in patients with colorectal cancer administered FOLFOX regimen,” Asian Journal of Surgery, vol. 44, no. 5, pp. 715–722, 2021.

[56] M. Payandeh, B. Shazad, M. Sadeghi, and M. Shahbazi, “Correlation between RAS test results and prognosis of metastatic colorectal cancer patients: a report from western Iran,” Asian Pacific Journal of Cancer Prevention, vol. 17, no. 4, pp. 1729–1732, 2016.

[57] N. Rahadiani, D. R. Handjari, M. Stephanie, and E. Krishnunni, “The low prevalence of colonic serrated adenocarcinoma with high KRAS mutational status at Cipto Mangunkusumo Hospital, Indonesia,” Medical Journal of Indonesia, vol. 27, no. 3, pp. 161–168, 2018.

[58] A. K. Siraj, R. Bu, S. Prabhakaran et al., “A very low incidence of BRAF mutations in Middle Eastern colorectal carcinoma,” Molecular Cancer, vol. 13, no. 1, 2014.

[59] Y. Song, L. Wang, W. Ran et al., “Effect of tumor location on clinicopathological and molecular markers in colorectal cancer in eastern China patients: an analysis of 2,356 cases,” Frontiers in Genetics, vol. 11, 2020.

[60] N. Saito, S. Tomita, K. Ichikawa, H. Mitomi, J. Imura, and T. Fujimori, “Analysis of KRAS mutations in cases of metastatic colorectal cancer at a single institution in Tochigi, Japan,” Pathobiology, vol. 81, no. 1, pp. 133–137, 2014.

[61] N. D. Sirisena, K. Deen, D. E. N. Mandawala, P. Herath, and V. H. W. Dissanayake, “The pattern of KRAS mutations in metastatic colorectal cancer: a retrospective audit from Sri Lanka,” BMC Research Notes, vol. 10, no. 1, p. 392, 2017.

[62] G. Segal, N. Liebermann, S. Klang et al., “Identification of KRAS mutations in colorectal cancer patients in Israel,” Harefuah, vol. 150, no. 5, pp. 447–450, 2011.

[63] H. Taniguchi, W. Okamoto, K. Muro et al., “Clinical validation of newly developed multiplex kit using Luminex xMAP technology for detecting simultaneous RAS and BRAF mutations in colorectal cancer: results of the RASKET-B study,” Neoaplasia, vol. 20, no. 12, pp. 1219–1226, 2018.

[64] V. H. Veldore, M. R. Rao, S. A. Prabhudesai et al., “Prevalence of KRAS mutations in metastatic colorectal cancer: a retrospective observational study from India,” Indian Journal of Cancer, vol. 51, no. 4, pp. 531–537, 2014.

[65] T. Watanabe, T. Kobunai, Y. Yamamoto et al., “Differential gene expression signatures between colorectal cancers with and without KRAS mutations: crosstalk between the KRAS pathway and other signalling pathways,” European Journal of Cancer, vol. 47, no. 13, pp. 1946–1954, 2011.

[66] T. Watanabe, T. Yoshino, H. Uetake et al., “KRAS mutational status in Japanese patients with colorectal cancer: results from a nationwide, multicenter, cross-sectional study,” Japanese Journal of Clinical Oncology, vol. 43, no. 7, pp. 706–712, 2013.

[67] A. Yari, A. Samoudi, A. Afzali et al., “Mutation status and prognostic value of KRAS and BRAF in Southeast Iranian colorectal cancer patients: first report from Southeast of Iran,” Journal of Gastrointestinal Cancer, vol. 52, no. 2, pp. 557–568, 2020.

[68] W. K. Yip, C. W. Choo, V. C. S. Leong, P. P. Leong, M. F. Jabar, and H. F. Seow, “Molecular alterations of Ras-Rafmitogen-activated protein kinase and phosphatidylinositol 3-kinase-Akt signaling pathways in colorectal cancers from a tertiary hospital at Kuala Lumpur, Malaysia,” Apmis, vol. 121, no. 10, pp. 954–966, 2013.

[69] T. Yoshino, K. Muro, K. Yamamoto et al., “Clinical validation of a multiplex kit for RAS mutations in colorectal cancer: results of the RASKET (RAS KEy Testing) prospective, multicenter study,” EBioMedicine, vol. 2, no. 4, pp. 317–323, 2015.

[70] A. Zahrani, M. Kandil, T. Badar, M. Abdelsalam, A. al-Faiar, and A. Ismail, “Clinico-pathological study of K-ras mutations in colorectal tumors in Saudi Arabia,” Tumori, vol. 100, no. 1, pp. 75–79, 2014.
[71] J. Zekri, M. A. Baghdadi, H. Alardati, H. Khalafil, and J. H. Kabanja, “Evaluation of the Idylla KRAS and NRAS mutation test in colorectal cancer tissue,” Experimental and Molecular Pathology, vol. 110, no. 10, 104270, 2019.

[72] J. Zekri, A. Rizvi, J. Al-Maghribi, and B. bin Sadiq, “K-ras in colorectal cancer tumors from Saudi patients: frequency, clinico-pathological association, and clinical outcome,” The Open Colorectal Cancer Journal, vol. 5, no. 1, pp. 22–27, 2012.

[73] J. Zhang, J. Zheng, Y. Yang et al., “Molecular spectrum of KRAS, NRAS, BRAF and PIK3CA mutations in Chinese colorectal cancer patients: analysis of 1,110 cases,” Scientific Reports, vol. 5, no. 1, 2015.

[74] L. Zhu, Y. Wang, Y. Zhou, Q. Dong, Y. Liu, and J. Zhang, “RAS mutational status detection in tissue, plasma, and stool samples for colorectal cancer,” BioMed Research International, vol. 2020, Article ID 5419634, 6 pages, 2020.

[75] X. Zhang, W. Ran, J. Wu et al., “Deficient mismatch repair and RAS mutation in colorectal carcinoma patients: a retrospective study in Eastern China,” PeerJ, vol. 2018, no. 2, 2018.

[76] Z. Zhihi Yong, G. T. H. Ching, and M. T. C. Ching, "Metastatic profile of colorectal cancer: interplay between primary tumor location and KRAS status," Journal of Surgical Research, vol. 246, pp. 325–334, 2020.

[77] R. Eachkoti, S. Farooq, S. I. Syeed, H. A. Wani, S. Majid, and H. Taniguchi, K. Uehara, G. Nakayama et al., "Prevalence and prognostic relevance of BrafV600E mutation in colorectal carcinomas from Kashmir (North India) valley," Mutagenesis, vol. 33, no. 3, pp. 225–230, 2018.

[78] X. Fu, H. Lin, X. Fan et al., "The spectrum, tendency and predictive value of PIK3CA mutation in Chinese colorectal cancer patients," Chinese Colorectal Cancer Patients, vol. 11, p. 868, 2021.

[79] J. Mohammadi Asl, S. Almasi, and M. A. Tabatabaiefar, "High frequency of BRAF proto-oncogene hot spot mutation V600E in cohort of colorectal cancer patients from Ahvaz City, Southwest Iran," Pakistan Journal of Biological Sciences, vol. 17, no. 4, pp. 565–569, 2014.

[80] L. S. Rozek, C. M. Herron, J. K. Greenson et al., "Smoking, gender, and ethnicity predict somatic BRAF mutations in colorectal cancer," Cancer Epidemiology and Prevention Biomarkers, vol. 19, no. 3, pp. 838–843, 2010.

[81] S. Saxena, V. Srinivas, P. Deb, D. K. Raman, and R. Jagani, "A study of BRAF mutation in colorectal carcinoma in Indian population," Journal of Cancer Research and Therapeutics, vol. 14, no. 6, pp. 1403–1406, 2018.

[82] Y. Shimada, Y. Tajima, M. Nagahashi et al., "Clinical significance of BRAF non-V600E mutations in colorectal cancer: a retrospective study of two institutions," Journal of Surgical Research, vol. 232, pp. 72–81, 2018.

[83] H. Taniguchi, K. Uehara, G. Nakayama et al., "Tumor location is associated with the prevalence of BRAF and PIK3CA mutations in patients with wild-type RAS colorectal cancer: a prospective multi-center cohort study in Japan," Translational Oncology, vol. 13, no. 7, article 100786, 2020.

[84] A. Vilkin, Y. Niv, T. Nagasaka et al., "Microsatellite instability, MLH1 promoter methylation, and BRAF mutation analysis in sporadic colorectal cancers of different ethnic groups in Israel," Cancer, vol. 115, no. 4, pp. 760–769, 2009.

[85] X. Wang, Q. Wei, J. Gao et al., "Clinicopathologic features and treatment efficacy of Chinese patients with BRAF-mutated metastatic colorectal cancer: a retrospective observational study," Chinese Journal of Cancer, vol. 36, no. 1, p. 81, 2017.

[86] M. I. Kusuma, J. A. Uwuratuw, E. Syarifuddin, and M. Faruk, "Relationship between BRAF V600E and KRAS mutations in stool for identifying colorectal cancer: a cross-sectional study," Annals of Medicine and Surgery, vol. 60, pp. 121–125, 2020.

[87] X. Zhang, J. Wu, L. Wang et al., "HER2 and BRAF mutation in colorectal cancer patients: a retrospective study in Eastern China," PeerJ, vol. 8, no. 2, p. e8602, 2020.

[88] J. H. Rho, J. J. Ladd, C. I. Li et al., "Protein and glycomic plasma markers for early detection of adenoma and colon cancer," Gut, vol. 67, no. 3, pp. 473–484, 2018.

[89] H. H. Hasbullah and M. Musa, "Gene therapy targeting p53 and KRAS for colorectal cancer treatment: a myth or the way forward?," International Journal of Molecular Sciences, vol. 22, no. 21, article 11941, 2021.

[90] J. Molina-Cerrillo, M. San Román, J. Pozas et al., "BRAF mutated colorectal cancer: new treatment approaches," Cancers, vol. 12, no. 6, p. 1571, 2020.

[91] J. Uhlig, M. Cecchini, A. Sheth, S. Stein, J. Lacy, and H. S. Kim, "Microsatellite instability and KRAS mutation in stage IV colorectal cancer: prevalence, geographic discrepancies, and outcomes from the National Cancer Database," Journal of the National Comprehensive Cancer Network, vol. 19, no. 3, pp. 307–318, 2021.

[92] K. Balschun, J. Haag, A. K. Wenke, N. T. Schwarz, and C. Röcken, "KRAS, NRAS, PIK3CA exon 20, and BRAF genotypes in synchronous and metachronous primary colorectal cancers: diagnostic and therapeutic implications," The Journal of Molecular Diagnostics, vol. 13, no. 4, pp. 436–445, 2011.

[93] F. Di Fiore, F. Blanchard, F. Charbonnier et al., "Clinical relevance of KRAS mutation detection in metastatic colorectal cancer treated by cetuximab plus chemotherapy," British Journal of Cancer, vol. 96, no. 8, pp. 1166–1169, 2007.

[94] L. Raskin, J. C. B. Dakubo, N. Palaski, J. K. Greenson, and S. B. Gruber, "Distinct molecular features of colorectal cancer in Ghana," Cancer Epidemiology, vol. 37, no. 5, pp. 556–561, 2013.

[95] S. A. Osasun, The pathological features of colorectal carcinoma in Ille-Ife—a ten-year descriptive retrospective study, Faculty of Pathology, 2007.

[96] G. Saffee Ardekan, S. M. Jafarnejad, L. Tan, A. Saeedi, and G. Li, "The prognostic value of BRAF mutation in colorectal cancer and melanoma: a systematic review and meta-analysis," PLoS One, vol. 7, no. 10, 2012.

[97] D. Chen, L. Q. Zhang, J. F. Huang et al., "BRAF mutations in patients with non-small cell lung cancer: a systematic review and meta-analysis," PLoS One, vol. 9, no. 6, article e101354, 2014.

[98] Z. Y. Yang, X. Y. Wu, Y. F. Huang et al., "Promising biomarkers for predicting the outcomes of patients with KRAS wild-type metastatic colorectal cancer treated with anti-epidermal growth factor receptor monoclonal antibodies: a systematic review with meta-analysis," International Journal of Cancer, vol. 133, no. 8, pp. 1914–1925, 2013.

[99] C. Therkildsen, T. K. Bergmann, T. Henrichsen-Schnack, S. Ladelund, and M. Nilbert, "The predictive value of KRAS, NRAS, BRAF, PIK3CA, and PTEN for anti-EGFR treatment..."
in metastatic colorectal cancer: a systematic review and meta-analysis," *Acta Oncologica*, vol. 53, no. 7, pp. 852–864, 2014.

[100] Y. J. Ma, X. L. Deng, and H. Q. Li, "BRAF V600E mutation and its association with clinicopathological features of papillary thyroid microcarcinoma: a meta-analysis," *Journal of Huazhong University of Science and Technology [Medical Sciences]*, vol. 35, no. 4, pp. 591–599, 2015.

[101] A. K. Arrington, E. I. Heinrich, W. Lee et al., "Prognostic and predictive roles of KRAS mutation in colorectal cancer," *International Journal of Molecular Sciences*, vol. 13, no. 10, pp. 12153–12168, 2012.

[102] D. Dinu, M. Dobre, E. Panaitescu et al., "Prognostic significance of KRAS gene mutations in the colorectal cancer-preliminary study," *Journal of Medicine and Life*, vol. 7, no. 4, p. 581, 2014.

[103] E. Domingo, R. Ramamoorthy, D. Oukrif et al., "Use of multivariate analysis to suggest a new molecular classification of colorectal cancer," *The Journal of Pathology*, vol. 229, no. 3, pp. 441–448, 2013.

[104] S. Ogino, K. Nosho, G. J. Kirkner et al., "PIK3CA mutation is associated with poor prognosis among patients with curatively resected colon cancer," *Journal of Clinical Oncology*, vol. 27, no. 9, p. 1477, 2009.

[105] A. I. Phipps, D. D. Buchanan, K. W. Makar et al., "KRAS mutation status in relation to colorectal cancer survival: the joint impact of correlated tumour markers," *British Journal of Cancer*, vol. 108, no. 8, pp. 1757–1764, 2013.

[106] M. Berg, S. A. Danielsen, T. Ahlquist et al., "DNA sequence profiles of the colorectal cancer critical gene set KRAS-BRAF-PIK3CA-PTEN-TP53 related to age at disease onset," *PloS One*, vol. 5, no. 11, article e13978, 2010.

[107] R. Nakanishi, J. Harada, M. Tuul et al., "Prognostic relevance of KRAS and BRAF mutations in Japanese patients with colorectal cancer," *International Journal of Clinical Oncology*, vol. 18, no. 6, pp. 1042–1048, 2013.

[108] G. A. Yanus, A. V. Belyaeva, A. O. Ivantsov et al., "Pattern of clinically relevant mutations in consecutive series of Russian colorectal cancer patients," *Medical Oncology*, vol. 30, no. 3, p. 686, 2013.

[109] L. Barault, N. Veyrie, V. Jooste et al., "Mutations in the RAS-MAPK, PI (3) K (phosphatidylinositol-3-OH kinase) signaling network correlate with poor survival in a population-based series of colon cancers," *International Journal of Cancer*, vol. 122, no. 10, pp. 2255–2259, 2008.

[110] J. Souglakos, J. Philips, R. Wang et al., "Prognostic and predictive value of common mutations for treatment response and survival in patients with metastatic colorectal cancer," *British Journal of Cancer*, vol. 101, no. 3, pp. 465–472, 2009.

[111] M. Zalis, F. M. Vieira, I. Zalberg-Renault, M. H. Bonamino, C. G. Ferreira, and S. Oliveira, "KRAS mutation profile in colorectal cancer patients in Brazil: a cohort of 989 individuals," *Journal of Clinical Oncology*, vol. 27, Supplement_15, pp. e15017–e15017, 2009.

[112] S. E. Baldus, K. L. Schaefer, R. Engers, D. Hartleb, N. H. Stoecklein, and H. E. Gabbert, "Prevalence and heterogeneity of KRAS, BRAF, and PIK3CA mutations in primary colorectal adenocarcinomas and their corresponding metastases," *Clinical Cancer Research*, vol. 16, no. 3, pp. 790–799, 2010.

[113] U. Miglio, R. Mezzapelle, A. Paganotti et al., "Mutation analysis of KRAS in primary colorectal cancer and matched metastases by means of highly sensitivity molecular assay," *Pathology-Research and Practice*, vol. 209, no. 4, pp. 233–236, 2013.

[114] L. Simi, N. Pratesi, M. Vignoli et al., "High-resolution melting analysis for rapid detection of KRAS, BRAF, and PIK3CA gene mutations in colorectal cancer," *American Journal of Clinical Pathology*, vol. 130, no. 2, pp. 247–253, 2008.

[115] C. Bozzao, D. Varvara, M. Pigilonaia et al., "Survey of KRAS, BRAF and PIK3CA mutational status in 209 consecutive Italian colorectal cancer patients," *The International Journal of Biological Markers*, vol. 27, no. 4, pp. 366–374, 2012.

[116] F. Selcukbiricik, S. Erdamar, C. U. Ozkurt et al., "The role of K-RAS and B-RAF mutations as biomarkers in metastatic colorectal cancer," *Journal of the Balkan Union of Oncology*, vol. 18, no. 1, pp. 116–123, 2013.

[117] N. Marchoudi, H. Amrani Hassani Joutei, F. Joulai, J. Fekkak, and H. Rhaisi, "Distribution of KRAS and BRAF mutations in Moroccan patients with advanced colorectal cancer," *Pathologie Biologie*, vol. 61, no. 6, pp. 273–276, 2013.

[118] A. Soliman, M. L. Bondy, S. A. el-Badawy et al., "Contrasting molecular pathology of colorectal carcinoma in Egyptian and Western patients," *British Journal of Cancer*, vol. 85, no. 7, pp. 1037–1046, 2001.

[119] A. O. Chan, A. S. Soliman, Q. Zhang et al., "Differing DNA methylation patterns and gene mutation frequencies in colorectal carcinomas from Middle Eastern countries," *Clinical Cancer Research*, vol. 11, no. 23, pp. 8281–8287, 2005.

[120] A. L. Schult, E. Botteri, G. Hoff et al., "Detection of cancers and advanced adenomas in asymptomatic participants in colorectal cancer screening: a cross-sectional study," *BMJ Open*, vol. 11, no. 7, article e048183, 2021.

[121] K. Heshmat-Ghahdarijani, J. Najafian, Z. Vafaei et al., "Rational, design and preliminary results of a cohort study on breast and colorectal cancer to develop a risk assessment model to predict future cardiovascular events. Cardiovascular events In Breast and Colorectal cancers (CIBC) study," *Current Problems in Cardiology*, no. article 100958, 2021 In press.

[122] T. G. Dolin, M. Mikkelsen, H. L. Jakobsen et al., "Geriatric assessment and intervention in older vulnerable patients undergoing surgery for colorectal cancer: a protocol for a randomized controlled trial (GEPOC trial)," *BMC Geriatrics*, vol. 21, p. 88, 2021.

[123] J. Ferlay, I. Soerjomataram, M. Ervik et al., "Estimated Cancer incidence, mortality and prevalence worldwide: sources, methods and major patterns in GLOBOCAN 2012," *International Journal of Cancer*, vol. 136, no. 5, pp. E359–E386, 2015.

[124] O. Majek, A. Gondos, L. Jansen et al., "Sex differences in colorectal cancer survival: population-based analysis of 164,996 colorectal cancer patients in Germany," *PloS One*, vol. 8, no. 7, article e68077, 2013.

[125] J. Thurmaier, V. Heinemann, J. Engel et al., "Changes in health-related outcomes among Western patients," *International Journal of Cancer*, vol. 136, no. 5, pp. E359–E386, 2015.
colorectal cancer patients undergoing inpatient rehabilitation therapy: a systematic review of observational and interventional studies,” *Acta Oncologica*, vol. 60, no. 1, pp. 124–134, 2021.

[128] K. F. Namiq, K. M. Ali, M. I. M. Gubari, and D. Asad, “Prevalence and predictive value of RAS mutations in metastatic colorectal cancer at Hiwa Cancer Hospital in Sulaimani Iraq,” *Pakistan Journal of Medical and Health Sciences*, vol. 14, no. 4, pp. 1485–1492, 2020.

[129] M. Ko, M. Garcia, C. Choi et al., “Microsatellite alterations with allelic loss at 9p24.2 signify less-aggressive colorectal cancer metastasis,” *Gastroenterology*, vol. 150, no. 4, pp. 944–955, 2016.

[130] H. Kawamata, K. Yamashita, K. Kojo, H. Ushiku, A. Ooki, and M. Watanabe, “Discrepancies between the K-ras mutational status of primary colorectal cancers and corresponding liver metastases are found in codon 13,” *Genomics*, vol. 106, no. 2, pp. 71–75, 2015.

[131] W. De Roock, B. Claes, D. Bernasconi et al., “Effects of KRAS, BRAF, NRAS, and PIK3CA mutations on the efficacy of cetuximab plus chemotherapy in chemotherapy-refractory metastatic colorectal cancer: a retrospective consortium analysis,” *The Lancet Oncology*, vol. 11, no. 8, pp. 753–762, 2010.

[132] M. Dobre, D. E. Dinu, E. Panaitescu et al., “KRAS gene mutations prognostic factor in colorectal cancer,” *Romanian Journal of Morphology and Embryology*, vol. 56, Supplement_2, pp. 671–678, 2015.

[133] E. K. Symvoulakis, A. Zaravinos, D. Panutopulos et al., “Highly conserved sequence of exon 15 BRAF gene and KRAS codon 12 mutation among Greek patients with colorectal cancer,” *The International Journal of Biological Markers*, vol. 22, no. 1, pp. 12–18, 2007.

[134] T. Ikoma, M. Shimokawa, M. Kotaka et al., “Clinical and prognostic features of patients with detailed RAS/BRAF-mutant colorectal cancer in Japan,” *BMC Cancer*, vol. 21, no. 1, pp. 1–10, 2021.

[135] C. G. Smith, D. Fisher, B. Claes et al., “Somatic profiling of the epidermal growth factor receptor pathway in tumors from patients with advanced colorectal cancer treated with chemotherapy+cetuximab,” *Clinical Cancer Research*, vol. 19, no. 15, pp. 4104–4113, 2013.

[136] T. Yokota, T. Ura, N. Shibata et al., “BRAF mutation is a powerful prognostic factor in advanced and recurrent colorectal cancer,” *British Journal of Cancer*, vol. 104, no. 5, pp. 856–862, 2011.

[137] M. Payandeh, N. Amirifard, M. Sadeghi et al., “The prevalence of KRAS mutation in colorectal cancer patients in Iranian population: a systematic review and meta-analysis study,” *Biomedical Research and Therapy*, vol. 4, no. 10, pp. 1693–1704, 2017.

[138] C. S. Lee, S. J. Baek, J. M. Kwak, J. Kim, and S. H. Kim, “Clinical characteristics of patients in their forties who underwent surgical resection for colorectal cancer in Korea,” *World Journal of Gastroenterology*, vol. 27, no. 25, p. 3901, 2021.

[139] S. Y. Kim, T. Kim, K. Kim, J. S. Bae, J. S. Kim, and C. K. Jung, “Highly prevalent BRAF V600E and low-frequency TERT promoter mutations underlie papillary thyroid carcinoma in Koreans,” *Journal of Pathology and Translational Medicine*, vol. 54, no. 4, pp. 310–317, 2020.

[140] E. Sanz-Garcia, G. Argiles, E. Elez, and J. Tabernero, “BRAF mutant colorectal cancer: prognosis, treatment, and new perspectives,” *Annals of Oncology*, vol. 28, no. 11, pp. 2648–2657, 2017.

[141] I. Lurkin, R. Stoehr, C. D. Hurst et al., “Two multiplex assays that simultaneously identify 22 possible mutation sites in the KRAS, BRAF, NRAS, and PIK3CA genes,” *PloS One*, vol. 5, no. 1, article e8802, 2010.

[142] J. G. Guedes, I. Veiga, P. Rocha et al., “High resolution melting analysis of KRAS, BRAF and PIK3CA in KRAS exon 2 wild-type metastatic colorectal cancer,” *BMC Cancer*, vol. 13, no. 1, pp. 1–10, 2013.

[143] F. Ciardiello, S. Tejpar, N. Normanno et al., “Uptake of KRAS mutation testing in patients with metastatic colorectal cancer in Europe Latin America and Asia,” *Targeted Oncology*, vol. 6, no. 3, pp. 133–145, 2011.

[144] S. Markowitz and M. J. N. E. J. M. Bertagnolli, “ESMO consensus guidelines for the management of patients with colon and rectal cancer. a personalized approach to clinical decision-making,” *Annals of Oncology*, vol. 23, no. 10, pp. 2479–2516, 2009.

[145] A. Sharma, N. R. Trivedi, M. A. Zimmerman, D. A. Tuveson, C. D. Smith, and G. P. Robertson, “Mutant V599EB-Raf regulates growth and vascular development of malignant melanoma tumors,” *Cancer Research*, vol. 65, no. 6, pp. 2412–2421, 2005.