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Prevalence of KRAS, PIK3CA, BRAF and AXIN2 gene mutations in colorectal cancer and its relationship with dental agenesis: a systematic review

Prevalencia de mutaciones en los genes KRAS, PIK3CA, BRAF y AXIN2 en cáncer colorrectal y su relación con agenesia dental: revisión sistemática

Running title: Agenesis and genetic variants prevalence

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Abstract
Introduction: The study of allelic and genotypic frequencies contributes to determining the distribution of genetic variants in different populations and their possible association with biomarkers. This knowledge could improve the decision-making process regarding the management of some diseases such as colorectal cancer (CRC), in which the detection of clinical biomarkers such as dental agenesis could be crucial in clinical practice.

Objective: To evaluate the available scientific evidence on the prevalence of \textit{KRAS}, \textit{PIK3CA}, \textit{BRAF} and \textit{AXIN2} mutations and their possible association with dental agenesis in people with CRC.

Materials and methods: A systematic search was conducted in PubMed, EMBASE and Cochrane Library databases using the following search strategy: type of studies: observational studies reporting the prevalence of \textit{KRAS}, \textit{PIK3CA}, \textit{BRAF} and \textit{AXIN2} mutations in people diagnosed with CRC and their possible association with dental agenesis; publication language: English and Spanish; publication period: 2010-2020; search terms: “Genes”, “RAS”, “Kras”, “PIK3CA”, “BRAF”, “AXIN2”, “Mutation”, “Polymorphism”, “Colorectal Neoplasms”, “Colorectal Cancer”, used in different combinations (“AND” and “OR”).

Results: The initial search yielded 403 records, but only 30 studies met the eligibility criteria. Of these, 11, 5, 5 and 1 only reported the prevalence of \textit{PIK3CA}, \textit{KRAS}, \textit{BRAF} and \textit{AXIN2} mutations, respectively;
while 8 reported the prevalence of more than one of these mutations in patients with CRC. The prevalence of KRAS (p.Gly12Asp), PIK3CA (p.Glu545Lys), and BRAF (p.Val600Glu) mutations ranged from 20.5% to 54%, 3.5% to 20.2%, and 2.5% to 12.1%, respectively. There were no findings regarding the association between the occurrence of these mutations and dental agenesis.

**Conclusions:** KRAS mutations were the most prevalent; however, there is no evidence on the association between dental agenesis and the occurrence of KRAS, PIK3CA and BRAF germline mutations in individuals with CRC.

**Keywords:** Anodontia; Mutation; Prevalence; Genes; Colorectal Cancer; Colorectal Neoplasms (MeSH).

**Sir-Mendoza F, Madera M, González-Martínez F.** Prevalence of KRAS, PIK3CA, BRAF and AXIN2 gene mutations in colorectal cancer and its relationship with dental agenesis: a systematic review. Rev. Fac. Med. 2023;71(1):e95595 (In Press). English. doi: [https://doi.org/10.15446/revfacmed.v71n1.95595](https://doi.org/10.15446/revfacmed.v71n1.95595).

**Resumen**

**Introducción.** El estudio de frecuencias alélicas y genotípicas contribuye a determinar la distribución de variantes genéticas en diferentes poblaciones y su posible asociación con biomarcadores. Este conocimiento podría mejorar la toma de decisiones respecto al manejo de algunas enfermedades como el cáncer colorrectal (CCR), en el cual la detección de biomarcadores clínicos como la agenesia dental podría ser crucial en la práctica clínica.

**Objetivo.** Evaluar la evidencia científica sobre la prevalencia de mutaciones KRAS, PIK3CA, BRAF y AXIN2 y su posible asociación con la agenesia
dental en individuos con CCR.

**Materiales y métodos.** Se realizó una búsqueda sistemática en PubMed, Embase y Cochrane Library empleando la siguiente estrategia de búsqueda: tipos de estudio: estudios observacionales que reportaran la prevalencia de mutaciones en los genes *KRAS*, *PIK3CA*, *BRAF* y *AXIN2* en personas con CCR y su posible asociación con agenesia dental; idioma: inglés y español; periodo de publicación: 2010-2020; términos de búsqueda: “Genes”, “RAS”, “Kras”, “PIK3CA”, “BRAF”, “AXIN2”, “Mutation”, “Polymorphism”, “Colorectal Neoplasms”, “Colorectal Cancer” en diferentes combinaciones (“AND” y “OR”).

**Resultados.** Se identificaron 403 registros, pero solo 30 cumplieron con los criterios de elegibilidad. De estos, 11, 5, 5 y 1 solo reportaron la prevalencia de mutaciones en *PIK3CA*, *KRAS*, *BRAF* y *AXIN2*, respectivamente, mientras que 8 reportaron la prevalencia de más de una de estas mutaciones en pacientes con CCR. La prevalencia de mutaciones en los genes *KRAS* (p.Gly12Asp), *PIK3CA* (p.Glu545Lys), y *BRAF* (p.Val600Glu) varió entre 20.5% y 54%, 3.5% y 20.2%, y 2.5% y 12.1%, respectivamente. No hubo hallazgos respecto a la asociación entre la ocurrencia de estas mutaciones y la agenesia dental.

**Conclusiones.** Las mutaciones de KRAS fueron las más prevalentes; sin embargo, no hay evidencia de la asociación entre agenesia dental y la ocurrencia de mutaciones en los genes *KRAS*, *PIK3CA* y *BRAF* en individuos con CCR.

**Palabras clave:** Anodoncia; Mutación; Prevalencia; Genes; Cáncer colorrectal; Neoplasma colorrectal (DeCS).

**Sir-Mendoza F, Madera M, González-Martínez F.** [Prevalencia de mutaciones en los genes *KRAS*, *PIK3CA*, *BRAF* y *AXIN2* en cáncer colorrectal.
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**Introduction**

Colorectal cancer (CRC) is one of the leading causes of morbimortality worldwide (1). According to demographic projections and temporal profiles, its global incidence is expected to increase by 60%, leading to more than 2.2 million new cases and 1.1 million deaths by 2030 (2). The cancer pathogenesis is complex and has not been completely understood. However, genetic factors reportedly play a critical role in tumorigenesis (3). Kolligs et al. reported that up to one third of the risk of developing CRC can be attributed to hereditary factors. Likewise, people whose families have a history of this cancer, could have a higher risk than those without this antecedent (4). Overall, genetic mutations are critical in the development of CRC. Therefore, several genes and signaling pathways have been related to this disease, such as *KRAS*, *BRAF*, *PIK3CA*, *RAS-RAF-MAPK* and *PI3K-PTEN-AKT* (5-7).

The main treatment approach for CRC is surgery and chemotherapy, which has an effectiveness of approximately 75%. However, roughly 30% of treated patients could develop new neoplastic polyps (8), suggesting that this treatment is not totally effective for this disease (9). Thus, new therapies have been proposed, developing anti-EGFR monoclonal antibodies drugs, considering that the Epidermal Growth Factor Receptor (EGFR) is the major therapeutic target in colorectal cancer (10). However, therapeutic effectiveness is affected in the presence of *KRAS*, *BRAF* and *PIK3CA* mutations (11,12). Thus, these genes constitute important biomarkers for CRC. In addition, it has been reported that mutations in
AXIN2 could act as a diagnostic biomarker for CRC associated to dental agenesis, so this gene variant and the presence of this oral developmental anomaly have been proposed as a predictive factor for this malignant disease (13).

Non-syndromic dental agenesis, the most common human anomaly (14), is the congenital absence of one or more permanent teeth due to alterations during early stages of dental development (15). Genetically, it has been described as the association between AXIN2 gene and teeth development in mice, suggesting its possible participation in human dental development (16). Lammi et al. reported the association between dental agenesis and the predisposition to CRC by a nonsense mutation (p.Arg656Stop) in AXIN2 (16). Likewise, Rosales et al. reported a higher risk of malignant CRC development in the presence of Single Nucleotide Variant (SNV) rs2240308 in the AXIN2 gene (17).

According to the above statements, it is important for all the healthcare professionals to know about the prevalence of these genetic mutations and their possible association with clinical biomarkers such as dental agenesis to improve the diagnosis, prognostic and early treatment of cancer. Particularly for dentistry practitioners, who have an important role in detecting dental agenesis, this process could promote possible medical diagnoses of CRC. Therefore, the aim of this study was to evaluate the available scientific evidence on the prevalence of KRAS, PIK3CA, BRAF, AXIN2 mutations and their possible association with dental agenesis in people diagnosed with CRC.

**Materials and Methods**

We conducted a systematic review following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (PRISMA)(18).
Search strategy
A structured and systematic search using MeSH and DeCS terms was performed in Medline (Via PubMed), EMBASE (Via Ovid) and Cochrane Library databases based on the following search strategy: type of studies: observational studies reporting the prevalence of KRAS, PIK3CA, BRAF and AXIN2 mutations in people diagnosed with CRC and their possible association with dental agenesis publication period: from January 2010 until September 2020; publication languages: English and Spanish; search terms: “Genes”, “RAS”, “Kras”, “PIK3CA”, “BRAF”, “AXIN2”, Prevalence”, “Mutation”, “Polymorphism”, “Colorectal Neoplasms”, “Colorectal Cancer”, and “dental agenesis”, used in different combinations (“AND” and “OR”). The search equation used in each database is shown in Appendix 1.

Studies screening and selection process
The titles and abstracts of the records retrieved in the searches were managed using the reference manager software EndNote® (Version X8, Thomson Reuters). After removing duplicates, two reviewers (FS and FG) independently screened all titles/abstracts to exclude articles that were not relevant for the objective of this systematic review. Then, screened articles were read in full text by the two reviewers to confirm if they addressed the topics of interest for this review and decide on their final inclusion for full analysis based on the following inclusion criteria: being case-control, cohort or cross-sectional studies addressing the prevalence of KRAS, PIK3CA, BRAF and AXIN2 mutations/polymorphisms in people diagnosed with primary (adenocarcinoma) or metastatic CRC and their possible association with dental agenesis. Furthermore, studies conducted in animals, those published before 2010, and those addressing other types of genetic alterations and reporting other associations with CRC or
in concomitance with other cancers were excluded.
Disagreements were resolved by consensus, and when necessary, a third reviewer (MM) participated in the discussion until an agreement was reached.

**Methodological quality assessment**

The methodological quality of the selected studies was evaluated independently by two appraisers following the criteria previously reported (19). Briefly, the evaluated criteria were a) question/aim of research (1 item), b) participants (5 items), c) comparability between groups studied (4 items), d) definition and measurement of the main variables (4 items), e) statistical analysis and confusion (4 items), global assessment of internal validity, f) results (4 items), g) conclusions, external validity and applicability of results (4 items), h) conflict of interest (1 item). Each item was assessed as “very good”, “good”, “regular”, “bad”, “not reported”, “not apply”, and global assessment of study quality, being qualified “high”, “medium” and “low”.

Two authors (FS and FG) determined a grading system with the scores “very good”, “good”, “regular”, “bad”, “not reported”, “not apply”, or “5”, “4”, “3”, “2”, “1”, “0”, respectively, with the highest total score being 135 and minimum of 27. The score articles within the interval 81 – 107 and regular internal validity were categorized with a “median” methodological quality, while a score over 108 represented a “high” methodological quality. However, in the articles that did not apply for the evaluation of criterion “c) comparability between the groups studied”, the maximum accumulated score was 115 and the minimum was 23. The score articles within the interval 69-91 and regular internal validity were categorized with a “medium” methodological quality, while a score of over 92 represented
a “high” methodological quality.

Data extraction and analysis
The following information was extracted for each study: author, publication year, geographic region in which the study was conducted, sample size, general prevalence of the mutation, mutation prevalence by sex (male and female), mutation (changes in amino acids) and sequencing techniques. The information was gathered in tables by genes.

Results
Selection and characterization of studies
The selection process is presented in Figure 1. In total, 30 articles were included in this systematic review for full analysis.
Source: own elaboration

Regarding their geographical distribution, 10 studies were conducted in Asia, 8 in America, 5 in the Middle East, 3 in Europe, 2 in Oceania and 1 in Africa. Regarding sample size, studies addressing KRAS, PIK3CA and
BRAF mutations were conducted in samples ranging from 49 to 5732, 61 to 2299, and from 17 to 1110 participants, respectively. Besides, cross-sectional studies were the most frequent type of study (100%) (Table 1).

**Table 1. General characteristics of included studies**

| Author (year)          | Region          | Study design | Studied sample | Genes assessed | MQA⁺ |
|------------------------|-----------------|--------------|----------------|----------------|------|
| Gavin PG. (2012)(20)   | United States   | CSS a        | 2299           | PIK3CA         | High |
| Palomba G. (2012)(21)  | Italy           | CSS          | 384            | PIK3CA         | High |
| Mao C. (2012)(22)      | China           | CSS          | 61             | PIK3CA         | High |
| Liao X. (2012)(23)     | United States   | CSS          | 1170           | PIK3CA         | High |
| Watanabe T. (2013)(24) | Japon           | CSS          | 5732           | KRAS           | High |
| Shen Y. (2013)(25)     | China           | CSS          | 674            | KRAS, BRAF     | High |
| Patil H. (2013)(26)    | India           | CSS          | 1323           | KRAS           | High |
| Chang YS. (2013)(27)   | Taiwan          | CSS          | 165            | KRAS, BRAF     | Median |
| Rosty C. (2013)(28)    | Australia       | CSS          | 757            | PIK3CA         | High |
| Kang M. (2013)(29)     | United States   | CSS          | 150            | PIK3CA         | Median |
| Marchoudi N. (2013)(30)| Morocco         | CSS          | 92             | BRAF           | High |
| Samadder NJ. (2013)(31)| United States   | CSS          | 563            | BRAF           | Median |
| Baskin Y. (2014)(32)   | Turkey          | CSS          | 49             | KRAS           | Median |
| Imamura Y. (2014)(33)  | United States   | CSS          | 1267           | KRAS           | High |
| Bader T. (2014) (34)   | Saudi Arabia    | CSS          | 83             | KRAS           | High |
| Chen J. (2014)(35)     | China           | CSS          | 214            | PIK3CA, KRAS,  | High |
| Bisht S. (2014)(36)    | India           | CSS          | 204            | PIK3CA         | High |
| Russo AL. (2014)(37)   | United States   | CSS          | 222            | PIK3CA         | High |
| Siraj AK (2014)(38)    | Saudi Arabia    | CSS          | 757            | BRAF           | High |

aCross Sectional Studies, ⁺Methodological Quality Assessment

Source: Own elaboration
Ye JX. (2015) (39)  China  CSS  535  KRAS, BRAF  Median
Zhang J. (2015)(7)  China  CSS  1110  KRAS, PIK3CA, BRAF  High
Phipps AL. (2015)(40) United States, Canada, Australia  CSS  377  PIK3CA  High
Foltran L. (2015)(41) Italy  CSS  194  PIK3CA  High
Allard MA (2015)(42) France  CSS  1428  BRAF  High
Vatandoust S. (2016) (43) Australia  CSS  3318  KRAS, BRAF  Median
Watson R. (2016)(44) United States  CSS  447  KRAS, BRAF  High
Al- Shamsi HO.(2016) (45) Arab countries  CSS  99  KRAS, PIK3CA, BRAF  High
Molaei M (2016)(46) Iran  CSS  85  BRAF  Median
Jauhri M. (2017)(47) India  CSS  112  PIK3CA  High
Chang SC. (2020)(48) Taiwan  CSS  161  AXIN2  High

Methodological quality assessment
Twenty-three studies scored “high” methodological quality, while 7 studies scored “medium” quality using the critical appraisal of epidemiological cross-sectional studies instrument (Table 1). The domains with the highest score were “participants” and “results”, whereas “statistical analysis and confusion” domain obtained the lowest score.

Mutation prevalence

**KRAS**
The higher overall prevalence was 54% in a sample of 447 individual and the lowest was 20.5% in a sample of 1323 individuals. The higher prevalence by sex was 66% and 50% for males and females, respectively. Moreover, 46% of the articles carried out direct sequencing and 23%, next generation sequencing (NGS) to identify mutations in **KRAS**. The most frequent mutation consisted of an amino acid change from glycine...
into aspartic acid in codon 12 (Table 2).

Table 2. Mutation of KRAS, PIK3CA, BRAF

| Author (year)          | Mutation (%) | Sex (Male) | Sex (Female) | Sequencing technique |
|------------------------|--------------|------------|--------------|---------------------|
| **KRAS**               |              |            |              |                     |
| Watanabe T. (2013)     | 37.6         | 35.5       | 40.9         | NR                  |
| Shen Y. (2013)         | 35.9         | 32.3       | 41.3         | G12D (13.6)         |
| Patil H. (2013)        | 20.5         | 20.3       | 20.8         | G12A (36.5)         |
| Chang YS. (2013)       | 36.9         | NR         | NR           | G12D (35.5)         |
| Baskin Y. (2014)       | 30.6         | 25.8       | 38.9         | G12D (12)           |
| Imamura Y. (2014)      | 40           | 50         | 50           | G12D (12)           |
| Bader T. (2014)        | 42.2         | 66         | 34           | G12D (45.7)         |
| **KRAS**               |              |            |              |                     |
| Chen J. (2014)         | 44.9         | 56.3       | 43.8         | G12D (35.4)         |
| Ye JX. (2015)          | 37.9         | 36.1       | 40.4         | G12D (18.4)         |
| Zhang J. (2015)        | 45.4         | 44.1       | 47.3         | G12D (40.7)         |
| Vatandoust S. (2016)   | 38.9         | NR         | NR           | NR                  |
| Watson R. (2016)       |              | NR         | NR           | PS, NGS,            |
| Al-Shamsi HO. (2016)   | 44.4         | 41.7       | 48.7         | NGS                 |
| **PIK3CA**             |              |            |              |                     |
| Gavin PG. (2012)       | 20.2         | 10.4       | 9.7          | NR                  |
| Palomba G. (2012)      | 17.4         | 16         | 19           | E545A (14)          |
| Mao C. (2012)          | 8.2          | 8.6        | 7.7          | H1047L (7)          |
| Liao X. (2012)         | 16           | 8.2        | 7.8          | NR                  |
| Rosty C. (2013)        | 14           | 50         | 50           | E542K (35)          |
| Kang M. (2013)         | 12           | NR         | NR           | NR                  |
| Chen J. (2014)         | 12.3         | 65.4       | 34.6         | H1047R (31)         |
| Bisht S. (2014)        | 5.9          | 5          | 7.1          | E545K (3.4)         |
| Russo AL. (2014)       | 13           | NR         | NR           | NR                  |
| Zhang J. (2015)        | 3.5          | 3.4        | 3.7          | H1047R (3.5)        |
| Phipps AL. (2015)      | 11           | NR         | NR           | E542K,E545K (3.5)   |
| Foltran L. (2015)      | 16.5         | NR         | NR           | E545K (56)          |
| Al-Shamsi HO. (2016)   | 13.1         | 13.3       | 12.2         | NR                  |
| Jauhari M. (2017)      | 16.1         | 22.1       | 6.8          | E545A, E545K, H1047R (15.8) |
Table 2 (cont.). Mutation of KRAS, PIK3CA, BRAF

| Author (year) | Mutation (%) | General (%) | Sex (%) | Mutation (%) | Sequencing technique |
|---------------|--------------|-------------|---------|--------------|----------------------|
|               |              | Male | Female |              |                      |
| **BRAF**      |              |      |       |              |                      |
| Chang YS. (2013)(27) | 4.2 | NR | NR | V600E (100) | HRM^i                |
| Shen Y. (2013)(25)  | 7.0 | 6.9 | 7.1 | V600E (1.8) | DS                   |
| Marchoudi N.(2013)(30) | 5.4 | NR | NR | V600E (100) | DS                   |
| Samadder NJ. (2013)(31) | 27 | NR | NR | V600E (100) | DS                   |
| Chen J. (2014)(35)  | 4.2 | 55.6 | 44.4 | V600E (89) | DS                   |
| Siraj AK. (2014)(38) | 2.5 | 2.8 | 2.2 | V600E (89.5) | DS                   |
| Allard MA. (2015)(42) | 6.4 | NR | NR | V600E (100) | HRM, DS              |
| Zhang J. (2015)(7)   | 3.1 | 2.8 | 3.5 | V600E (100) | DS, ARMS, NGS        |
| Ye JX. (2015)(39)    | 4.4 | 2.8 | 6.6 | V600E (80) | DS, ARMS             |
| Vatandoust S. (2016)(43) | 12.1 | NR | NR | NR | NR                   |
| Al-Shamsi HO. (2016)(45) | 4.0 | 3.3 | 5.1 | NR | NGS                  |
| Molaei M. (2016)(46) | 0 | 0 | 0 | NR | DS                   |
| Watson R. (2016)(44) | 0 | 0 | 0 | NR | PS, NGS              |
| **AXIN2**        |              |      |       |              |                      |
| Chang SC. (2020)(48) | 21.7 | NR | NR | A603P (11.4) | NGS                  |

- Direct Sequencing, ^Luminex Assay, ^c Primer Extension Assay,
- Array Analysis, ^d Amplification Refractory Mutations System-PCR, ^e Pyrosequencing, ^f Next Generation Sequencing, ^h Not reported, ^i Single-nucleotide primer extension, ^j High Resolution Melting

Source: Own elaboration

**PIK3CA**

The higher general prevalence was 20.2% in a studied sample of 2299
and the lowest was 3.5% in a sample of 1110 individuals. The higher prevalence according to sex was 65.4% for male and 50% for female. The 57% of selected articles carried out direct sequencing and 21.4% next generation sequencing (NGS). The most frequent mutation was the substitution of glutamic acid by lysine in codon 545 (p.Glu545Lys) (Table 2).

**BRAF**

The higher general prevalence was 12.1% in a studied sample of 173 and lowest was 2.5% in 757 individuals. Two studies did not obtain mutations in their samples. The higher prevalence according to sex was 55.6% and 44.4% for male and female, respectively. The 69% of selected articles carried out direct sequencing and 23% next generation sequencing (NGS). The most frequent variant was an aminoacid change of valine by glutamate in codon 600 (p.Val600EGLu) (Table 2).

**AXIN2**

Only one study (48) was selected with a general prevalence of 21.7% in a sample from Taiwan. The variant p.A603P was the most frequently detected through a next generation sequencing platform (Table 2).

**Discussion**

The fact that cancer cells contain multiple genetic mutations suggests that the development and progression of tumors could be in part caused by mutagenesis. Additionally, these events can contribute to develop resistance to conventional oncological therapies, such as chemotherapy (49). Currently, scientific evidence shows that therapy against cancer is limited, despite new drug developments, since only new ways of resistance have emerged, such as inactivation and drug output, alteration of therapy targets and inhibition of cell death (50). In relation to the foregoing
statements, it is undoubtedly essential to understand the distribution of mutations in oncogenes in cancer patients to contribute to the knowledge of the genomic profile of malignant diseases and personalized medicine. Consequently, these contributions have currently allowed the understanding of the cancer genome, which has become in an important aspect for clinical decisions to select the best treatment available for each oncological patient (51).

The existing literature reports mutations in multiple genes involved with development and progression of colorectal cancer, among them and with a higher prevalence, KRAS, PIK3CA and BRAF. In addition, a gene has been identified that is currently important for its possible usefulness in the early diagnosis of colorectal cancer through clinical markers such dental agenesis, and it is named AXIN2 (16).

KRAS mutations are the most prevalent events in the development of human tumors (52,53). KRAS encodes for a protein constituted by 188 residues of amino acids, implicated in molecular pathways activation, allowing transduction signals from the cell surface to the nucleus (54). KRAS is found in chromosome 12 and is a member of the RAS family; it comprises 86% of all family RAS mutations. The most frequent mutations observed are in codons 12 and 13 of exon 1 (55), and less frequently, in codons 61 (56) and 146 (57,58). The main variant consists in a G>A transition followed by a G>T transversion in exon 1 (59). KRAS has been studied as a predictive molecular marker against anti-EGFR in primary and metastatic colorectal cancer (11, 60, 61). In the presence of mutations in KRAS, GTPase activity decreases and the KRAS mutant protein remains bound to GTP in its active conformation, transmitting signals continuously.
As a result, signal transmission is not blocked by anti-EGFR and therapeutic effects are scarce or could not be observed (62-64).

In this study, a range of 20.5% to 54% was obtained for mutation prevalence for *KRAS* (7,24-27,32-35,39,43-45). Regarding the geographic distribution of these genetic variants, the highest prevalence reported in America was 54% in a sample of 447 individuals (44). In contrast, in the same region, the lowest prevalence was 40% in 1267 individuals (33). In relation to sex, a prevalence of 50% was reported for males and females (33). In Asian countries, the highest prevalence was 45.4% in 1110 Chinese individuals (7), while the lowest was 20.5% in India, in a sample of 1323 (26). By sex, 56.3% was the highest prevalence for males (35) and 47.3% for females (7). In the Middle East, the highest prevalence was 44.4% in a sample of 99 individuals from several countries (45) compared to 30.6%, the lowest prevalence having been found in a sample of 49 individuals from Turkey (32). Regarding sex, 66% was the highest prevalence for males (34) and 48.7% for females (45). In Oceania, only one article was selected in this study, reporting a prevalence of 38.9% in a study sample of 778 Australian subjects (43). By sex, no data were reported (43). The most frequent mutation in KRAS was the change of glycine by aspartate (p. Gly12Asp). However, one study reported a higher prevalence concerning the change of glycine by alanine (p. Gly12Ala) (26). These conformational biochemical changes have been associated with a poor survival prognostic and a rise in tumoral aggressiveness (32,65,66).

*PIK3CA* is found in chromosome 3 and encodes for PI3K protein. PI3K is part of the lipid kinases family, implicated in the proliferation, morphology, and cellular survival (67, 68). PI3K is involved in the PI3K/AKT pathway
which catalyzes AKT phosphorylation, activating the downstream signaling pathway (69). The mutated gene stimulates the pathway and promotes cell growth in various types of cancers (70). The prevalence of PIK3CA mutations have been reported for 15% to 20% of cases of colorectal cancer with the most frequent transition being G>A in exon 9 and 20. These hotspots regions comprise 80% of mutations of the whole gene (71, 28, 72), of which, those present in exon 20 are related to a low response to treatment with cetuximab and chemotherapy (73). On the other hand, analyses of mutations in this gene reportedly could not contribute to improving the prediction of the response to monoclonal therapy with cetuximab (74).

Regarding the prevalence of mutations in PIK3CA, an interval of 3.5% to 20.2% was obtained (7,20-23,28,29,35,36,37,40,41,45,47). Considering the geographic distribution of these prevalences, in America, 20.2% was the highest prevalence in a studied sample of 2299 individuals in the United States (20). Conversely, the lowest (11%) was reported in a sample of 377 in individuals from the United States and Canada (40). Regarding sex, 10.4% and 9.7% were the highest prevalences for males and females, respectively (20). In Asian countries, the highest prevalence was reported in India with 16% in a sample of 112 (47), and the lowest was 3.5% in Chinese subjects (7). Similarly, 65.4% and 34.6% were the highest prevalences for male and female, respectively (35). In the Middle East, only one article was selected, reporting a prevalence of 13% (45). Regarding sex, 13% for males and 12% for females, in a sample of 99 individuals from Middle Eastern countries (45). In western Europe, 17.4% was the highest prevalence (21), and regarding sex, the reported prevalences were 16% and 19% for males and females, respectively, in a
sample of 384 Italian individuals (21). In Oceania, one study was chosen, reporting a prevalence of 14% (28). Regarding sex, a prevalence of 50% was found for both sexes in a sample of 757 Australian individuals (28).

The most frequent variant in the PIK3CA leads the change of glutamic acid into lysine (p.Glu545Lys) due to alterations in exon 9. However, a high mutation index was also reported in exon 20, resulting in a change of histidine by leucine (p.His1047Leu) and histidine by arginine (p.His1047Arg).

BRAF intervenes in the proliferation, differentiation, and cell apoptosis pathways (75), indicating that significant alterations in this gene could lead to phenotypic alterations in colorectal tissue. In this study, the prevalence of mutations in BRAF has been reported as ranging from 2.5% to 27% (7,25,27, 30,31,35,38,39,42-46). Regarding the geographical distribution of these mutations, in America, the highest prevalence was 27% in a sample of 563 individuals (31). In contrast, Watson R et al. did not report the presence of mutations in BRAF in 17 individuals (44), both studies being conducted in the United States. Concerning sex, no data was reported (44). In Asia, the highest prevalence was 7% in a sample of 674 (25) and the lowest was 3.1% in 1110 individuals (7), both studies conducted in China. In relation to sex, 55.6% and 44.4% were the highest prevalences for males and females (35), respectively. In the Middle East, the highest prevalence reported was 4% in a sample of 99 individuals from several countries (45). In contrast, Molaei M et al. do not report the presence of mutations in BRAF in 85 Iranian individuals studied (46). According to sex, the highest prevalence for males was 3.3%, and 5.1% for females (45). In Western Europe, the highest prevalence reported
was 6.4% in 1428 individuals studied in France (42). In Africa, 5.4% was the highest prevalence in a sample of 92 Moroccan individuals (30). In Oceania, the highest prevalence was 12.1% in a sample of 173 individuals in Australia (43). In the last three regions, no prevalence was reported in relation to sex. The most frequent mutation was the change of valine by glutamate in codon 600 (p. Val600Glu). Samowitz et al. reported that individuals with mutations in \textit{BRAF} have more aggressive colorectal cancer phenotypes and no positive prognostic with cetuximab or panitumumab (76). Consequently, it is recommended to expand the study spectrum of other possible hotspot regions in \textit{BRAF} associated with this disease, because most of the selected studies only studied the genetic variant corresponding to p.Val600Glu.

In comparison with studies before 2012, regarding the \textit{KRAS} gene, Segura et al. reported a general prevalence of 32.4% in 37 colorectal tumors in Mexican individuals (77). Vaughn et al. obtained a general prevalence of 42.4% in 2121 colorectal adenocarcinomas in the United States (78). In relation to \textit{PIK3CA}, Herreros-Villanueva et al. (79) and Velho et al. (80) reported prevalences of 8.22% and 7.1%, in samples of 73 Spanish patients and 103 colorectal cancer specimens, respectively. Regarding \textit{BRAF}, Di Nicolantonio et al. reported a prevalence of 14% in 113 individuals with metastatic colorectal cancer in Italy and Switzerland (11). These comparisons could suggest that the frequencies of these genetic variants have remained within the ranges of general prevalence of mutations obtained in articles of later years reported in the present study. However, systematic reviews that include a greater range of years to evaluate increasing or decreasing trends of mutation prevalence in these genes over time are necessary.
The differences between general prevalences of mutations and prevalences according to sex in the same geographical area could be attributed to differences in the size of the samples studied and to the sensitivity of the molecular techniques used, which have been shown to influence the frequency mutation detection (81,82). Other factors that could have an influence are the quality and quantity of the DNA obtained, the heterogeneity in the tumor and possible environmental exposures not controlled or unknown by the authors (83,84).

Although the relationship between the prevalence of the studied genetic mutations and sex was not statistically significant in most of the articles reported in the present study, this relationship was evaluated because it has been reported that genetic associations with sex could provide information about the pathogenesis of diseases (85), and also because of the established differences in susceptibility and incidence of cancer among men and women around the world (86), attributable to environmental causes and genetic differences (87, 88). Therefore, it can be thought that differences in the prevalence of mutations between males and females could condition the development of colorectal cancer more frequently in individuals of a specific sex.

The incidence and mortality of colorectal cancer has increased over the past 10 years (89), therefore, the need to identify and implement early diagnostic strategies for this malignant pathology has increased, including the analysis of molecular and clinical biomarkers. Recently, a gene that could be considered a molecular biomarker, AXIN2, has been studied and a possible association of variants in this gene, as well as the phenotype of colorectal cancer and dental agenesis have been reported (16). The
present systematic review aims to report the prevalence of variants in \textit{AXIN2} in individuals with primary and/or metastatic colorectal cancer around the world. However, only one study was identified. In addition, due to the knowledge of the possible relations of the \textit{AXIN2} gene with both phenotypes, we attempted to identify if some of the higher prevalent genes in colorectal cancer such as \textit{KRAS}, \textit{PIK3CA} and \textit{BRAF} were also related to dental agenesis, however, no studies were identified that reported such an association.

\textit{AXIN2} is known for its tumor suppressing activity by negatively regulating the WNT pathway by the intracellular degradation of β-catenine (90,91). In mice, \textit{AXIN2} is expressed during odontogenesis in dental mesenchyme, enamel knot, dental papilla and mesenchymal odontoblast. It is reasonable to hypothesize that a loss in function of this gene could affect the development of molars and incisors, leading to dental agenesis (92). In addition, there is evidence that the expression of \textit{AXIN2} in colorectal tissue can lead to carcinomas (16). Wu Z \textit{et al.} reported that mutations in \textit{AXIN2} could influence the expression of its protein, which can play a critical role in carcinogenesis (93), being similar to reports by Rosales \textit{et al.}, who claims that these mutations act as a genetic risk factor for the development of colorectal cancer (17). Marvin L \textit{et al.} reported the nonsense mutation p.Tyr663X (c.1989G> A) in \textit{AXIN2}, which generates a truncated protein in individuals with oligodontia, gastrointestinal neoplasms and other clinical manifestations (94). Therefore, dental agenesis and variants in \textit{AXIN2} could possibly be used as clinical and molecular markers for susceptibility in colorectal cancer development.

The present study highlights the importance of investigating the distribution
of mutations in KRAS, PIK3CA, BRAF and AXIN2 in individuals with colorectal cancer in different populations around the world to determine the impact of these variants in the early diagnosis, prognosis, survival and therapeutic effectiveness in individuals with this malignant pathology. It is important to study the genetic mutations in heterogeneous populations, due to the higher probability of mortality and poor prognosis of colorectal cancer that could be related to ethnic and sexual differences regarding the presence of certain genetic variants. Ethnicity has been related to risk (93, 95-97) and a worse prognosis of cancer in the presence of KRAS (98), PIK3CA (99) and BRAF (100) mutations.

Additional studies of genetic associations, including KRAS, PIK3CA, BRAF and AXIN2 with colorectal cancer are suggested in diverse populations to contribute to the knowledge on the cancer genome. Likewise, studies focused on the relationship between variants in AXIN2 and dental agenesis are recommended as an early clinical marker of colorectal cancer.

**Conclusions**

Our findings suggest that there is a wide and diverse distribution of KRAS, PIK3CA and BRAF gene mutations in individuals with CRC worldwide; KRAS being the most prevalent. Moreover, this study highlights that there is no evidence on the association between dental agenesis and KRAS, PIK3CA and BRAF germline gene mutations in people with CRC. AXIN2 is the unique gene in which the association with both phenotypes has been well stated, but population studies focused on AXIN2 mutations prevalence are limited.

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Appendix 1. Search equations:

**MEDLINE (via PubMed)**

(“Prevalence” AND (“Mutation” OR “Polymorphism”) AND (“Genes” OR “RAS” OR “Kras”) AND (“Colorectal Neoplasms” OR “Colorectal Cancer”) AND “dental agenesis”)

(“Prevalence” AND (“Mutation” OR “Polymorphism”) AND (“Genes” OR “PIK3CA”) AND (“Colorectal Neoplasms” OR “Colorectal Cancer”) AND “dental agenesis”)

(“Prevalence” AND (“Mutation” OR “Polymorphism”) AND (“Genes” OR “BRAF”) AND (“Colorectal Neoplasms” OR “Colorectal Cancer”) AND “dental agenesis”)

(“Prevalence” AND (“Mutation” OR “Polymorphism”) AND (“Genes” OR “dental agenesis”))
“AXIN2”) AND (“Colorectal Neoplasms” OR “Colorectal Cancer”) AND “dental agenesis”

**Embase (via Ovid)**

((“Mutation” OR Polymorphism*) AND “Prevalence” AND (“RAS” OR “Kras”) OR “PIK3CA” OR “BRAF” OR “AXIN2” AND (“Colorectal Neoplasms” OR “Colorectal Cancer”) AND “dental agenesis”)

**Cochrane Library**

(“Prevalence” AND (“Mutation” OR “Polymorphism”) AND (“Genes”) AND (“Colorectal Neoplasms” OR “Colorectal Cancer”) AND “dental agenesis”)