INTRODUCTION

Dental agenesis is one of the most common congenital anomalies in human dentition. Hypodontia occurs when there are one to five missing teeth and its prevalence reaches 2.6–11.3% depending on the ethnic group.1 Although the etiology of dental agenesis involves genetic and environmental factors, the genes more frequently associated with hypodontia in different populations are AXIN2, MSX1, PAX9, EDA, and WNT10.2,3

From the study of Lamni et al.,4 many investigations relating dental agenesis with predisposition to cancer, primarily colorectal cancer (CRC), have arisen.5–7 This association is supported by the molecular events that keep homeostasis of morphogenesis and tissue regeneration. CRC is the fourth most common cause of cancer-related deaths in the world8 and it has been reported that colorectal carcinogenesis is associated with alterations in Wnt signaling. Some CRC, like adenomatous polyposis and hereditary nonpolyposis CRC syndrome (Lynch syndrome), involve mutations in germline or in repair genes.9 Developmental homeostasis involves the wingless/integration (WNT) signaling pathway controlling cell proliferation, differentiation, and cell death.10 When cells receive the WNT signal, β-catenin is stabilized and joins the DNA-bound T-cell factor family of transcription proteins for regulating the expression of target genes. In the absence of WNT, β-catenin protein is degraded by the proteasome via action of a multiprotein complex. This complex is composed of the tumor suppressor adenomatous polyposis coli gene product (APC) and AXIN1 (axis inhibition protein 1) or its homologous protein, AXIN2. They formed a structure with β-catenin, glycogen synthase kinase 3B (GSK3β), and disheveled (DLV) protein.11 Hence, molecules such as WNT (WNT4, WNT6, and WNT10) and AXIN2 play an important role during the embryonic development that involves dental formation.12

The association between AXIN2 and CRC involves defects in the canonical WNT signaling pathway, which regulates and coordinates the AXIN complex for the degradation of β-catenin under normal conditions. In addition, AXIN2 expression can be elevated in CRC as a result of APC mutations. However, and in an independent way, alterations in AXIN2 (loss-of-function, dosage dependent, or even gain-of-function mutations) can contribute to development of gastric cancer.13

The genetic connection between alterations in embryonic development of dental organs and predisposition to cancer is understandable; particularly the finding that AXIN2 mutations could lead to an inefficient block of the WNT signaling pathway. Somatic mutations in the AXIN-complex proteins associated with degradation of β-catenin or mutations in β-catenin have been found in different tissues with carcinoma, including skin, gastrointestinal, hepatocellular, and ovarian epithelial cancer.6,14 In addition, AXIN2 has also been independently associated with tooth agenesis and non-syndromic cleft lip palate (NSCLP).15

AXIN2 SNP rs2240308 has been mapped at human chromosome 17q23-q24. This polymorphism (rs2240308, c.148 G>A) results in an amino acid change from proline to a serine. Although, rs2240308 has been associated with hypodontia and cancer in different populations,16,17 in Iranian subjects, this polymorphism was related with decreased risk for CRC.18 The inconsistent results
reported in literature are explained by racial differences.\textsuperscript{17} In Latino populations, genetic studies relating hypodontia and CRC are scarce, although AXIN2 rs2240308 was recently associated with CRC in Mexican population.\textsuperscript{19} Therefore, identifying and analyzing genetic mutations in CRC and hypodontia can provide relevant information about the biological behavior of both diseases. The aim of this study was to identify the association between AXIN2 rs2240308 with hypodontia and CRC.

MATERIALS AND METHODS
Population sample
The population consisted of individuals who assisted to Dentistry faculties at Pontificia Universidad Javeriana and Universidad de Cartagena and adults who underwent surgery for CRC at gastroenterology private clinic in Bogotá, and Hospital Universitario del Caribe in Cartagena, Colombia. Subjects were selected randomly from these institutions and population sample was divided into three groups according to their pathology. A group of 50 subjects with hypodontia, a group of 50 subjects with CRC, and a control group of 155 healthy individuals from both cities in Colombia. This observational analytical cross-sectional study was approved by the Ethical Committees of Dentistry faculty of Pontificia Universidad Javeriana (CIEFOUJ 201108-7539). Informed consent was obtained from all subjects participating.

Patients with hypodontia and healthy individuals were examined at the same clinic by two dentists and patients with CRC were operated by the single professional. Hypodontia-affected individuals were in the age range of 18–28 years. Hypodontia diagnosis was confirmed through complete intraoral examination, panoramic radiographies, and clinical records. Subjects with an uncertain hypodontia diagnosis, syndromes associated with hypodontia, trauma history, or agensis of third molars were excluded from the study. Subjects with CRC were in the age range of 32–64 years. CRC in all patients was histopathologically confirmed. There was no restriction on sex, age, or histopathological classification and states for selection of CRC patients. The control group included age, sex, and ethnic background matched selected from healthy individuals in the same area during the same time period as the case study. The ethnic background was determined by skin pigmentation and origin of the participants. Subjects with syndromes, hypodontia, trauma, or any type of cancer were excluded.

Genotyping
Self-reported family history of cancer and hypodontia was collected through questionnaire in all participants (control hypodontia and CRC groups). DNA was obtained from saliva samples through Oragene\textsuperscript{®} DNA kit (DNA Genonc Inc, Canada). AXIN2 rs2240308 PCR products were obtained from the samples of all the individuals enrolled in the study and sent for genotyping to the Molecular Cloning Laboratories (MC Lab, San Francisco, CA).

In silico analysis
With the aim of defining the genomic profile of AXIN2 in CRC, we performed an in silico analysis on data obtained from the web resource cBioPortal. Three comprehensive studies and one TCGA Provisional study were considered: DFCI, Cell Reports 2016 \(n = \) 619, Genentech, Nature 2012 \(n = \) 276, TCGA, Provisional (raw data at the NCI, \(n = \) 633), and MSK, Cancer Cell 2018 \(n = \) 1134).\textsuperscript{20–22} The tools provided by cBioportal and R packages from Bioconductor were employed for data integrative analysis and visualization.

For each study, we obtained a network of the most frequently altered neighboring genes of AXIN2. The number of genes was filtered according to the percentage of alteration of the neighbor genes, with a set threshold >2.6\%. This threshold was established in 2.6 because it is the minimum threshold for some altered gene associated with AXIN2 to appear, at least in the study of Cell Reports 2016. The same procedure was applied to each study, we set the lower threshold in order to obtain altered genes related with AXIN2. To get the overlapping genes between the networks, Euler Diagram was used. APC was the most redundant gene. For gene network association and functional enrichment analysis, we employed the web resources GeneMania (https://genemania.org/) and Enrichr (http://amp.pharm.mssm.edu/Enrichr/), respectively.

RESULTS
Genotyping
The demographic data for the study participants are described in Table 1. Familial cancer history was higher in patients with hypodontia compared with the control group showing a statistical significant difference for AXIN2 rs2240308 \(p < 0.005\) OR = 1.75, 95% CI: 1.22–6.91). However, Gastric cancer was the most frequent cancer in relatives of patients with hypodontia. In contrast, only 10% of patients with CRC reported family history of hypodontia (Table 2).

The observed genotype distribution for the Axin2 rs2240308 polymorphism in all groups, controls, hypodontia, and CRC was in agreement with the Hardy–Weinberg equilibrium. Statistical significant association was observed for CRC with AXIN2 rs2240308 \(\text{OR} = 5.4, 95\% \text{CI}: 2.7–10.4; p < 0.001\). The most frequent type and stage of CRC in this sample was adenocarcinoma (68%) and Dukes’B colon cancer (63%).

Regarding to hypodontia, there was no significant association with AXIN2 rs2240308 \(p < 0.31\). The most frequent tooth agenesis was observed in upper lateral incisors (40%) followed by mandibular second premolars (24%). Other teeth with agenesis were lower lateral incisors, maxillary first premolars, and lower central incisors (36%).

| Table 1. Phenotype for the study participants |
|--------------------------------------------|
| **Phenotype** | **CRC group** | **Hypodontia group** | **Control group** |
| Age (years) | 32–64 | 18–28 | 18–64 |
| Sex | | | |
| Male | 37 | 23 | 77 |
| Female | 13 | 27 | 78 |
| Ethnic background | | | |
| American | 29 | 29 American | 109 American |
| European | 12 | European | 27 European |
| Colombia (Latin America) | 8 | 129 American, | 27 European |
| Origin (place of birth and residence) | 25 | 25 Cartagena | 65 Cartagena |
| Cartagena (Caribbean) | 25 Cartagena (Caribbean) | 65 Cartagena (Caribbean) |
| Bogotá (Central) | 25 Bogotá (Central) | 90 Bogotá (Central) |

Cartagena is located at Caribbean region and Bogotá is located in central zone of Colombia.
In silico analysis
The genomic profile of AXIN2 in CRC patients indicates that the frequency of alteration/mutation of the gene is usually not higher than 10% of the patients. It is commonly associated with the alteration of other genes related with the WNT pathway, such as APC and CTNNB1. Genomic profile for AXIN2 in CRC is shown in Figs. 1 and 2. Also, there was an association with the tumor location, being AXIN2 gene more frequently mutated in tumor samples derived from the right colon than those derived from the left. Staging and sample type were also evaluated and the group of AXIN2 mutated showed an association with earlier stages compared with the other group. Interestingly, overall survival analysis indicated that patients who carry variations in the AXIN2 gene have a worse prognosis ($p < 0.05$).

In summary, by data mining analysis we have defined the genomic profile of AXIN2 gene in CRC. It is altered in 5–10% of CRC patients, it would be associated with the MSI molecular subtype and right-side tumors. Moreover, AXIN2 was found more frequently altered in early-stage tumors compared with metastatic CRC. However, patients who harbor mutations in AXIN2 were found to be associated with a worse prognosis. Along with AXIN2 those patients also showed mutations in WNT pathway related genes such as APC, RNF43, PIK3CA, among others. Mutations in these genes, as well the activation of the WNT pathway, have been primarily associated with Instability Microsatellite (MSI) molecular subtype right-side CRC tumors. It is possible that AXIN2 mutation can be a passenger of these driver genes in MSS tumors, but in turn it could be considered a driver gene in MSI right-side tumors. The association of AXIN2 mutation with poor prognosis and its appearance in early stages, position it as a prognostic and predictive marker in the defined molecular subtype of right-side colorectal tumors with MSI.

**DISCUSSION**
The association between AXIN2 and CRC has been demonstrated in different populations, but the association between rs2240308...
and CRC in Latino American population, it has been previously reported only in Mexican population. The present study showed a statistically significant association between AXIN2 rs2240308 and CRC (OR = 5.4 CI: 2.7–10.4; p < 0.001). Latin America has a history of large admixture between Africans, Europeans, and Native Americans, for this reason, this region has a high physical and genetic ancestry variation. The Asian ancestry in Colombia and Mexico is <1%. Significant differences have been reported in the association between rs2240308 and the risk of cancer for type of cancer and ethnic group. Then, while it has been reported that rs2240308 increased the risk of lung cancer especially in Asian population, a recent analysis indicated that AXIN2 148 C > T (rs2240308) variant may be associated with decrease lung risk in Asian and Caucasian populations. Therefore, further studies in Latin American population should be conducted to explain the association between AXIN2 polymorphisms and CRC.

Literature about the association of AXIN2 rs2240308 polymorphism with cancer show inconsistent results. AXIN2 rs2240308 polymorphism has been mainly associated with prostate and lung cancer, but its association with ovarian cancer, head and neck cancer, astrocytoma, and CRC did not show similar results. Liu et al. reported that AXIN2 is overexpressed in CRC in patients with DNA mismatch repair, but in this study, they did not report the association with rs2240308. In addition, several studies propose that hypodontia associated with AXIN2 variations could be a risk marker for CRC. In contrast, other studies propose Axin2 rs2240308 as a potential therapeutic target for preventing tumor growth. The possible explanations for these inconsistencies are related with racial differences observed in these associations, or, with other gene interactions and gene pathways involved in CRC and in tooth development.

Other possible explanation could be related with the results shown in silico analysis. This analysis demonstrated that the mutation of AXIN2 observed in CRC is usually not higher than 10% of the patients and it is commonly associated with the

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**Fig. 2** Comprehensive analysis of AXIN2 genomic profile on data from the study of Yaeger et al. a AXIN2 mutated samples in the study of Cancer Cell 2018, a total of 53 out of the 1099 patients harbored at least one mutation in AXIN2 gene. b Association of clinical variables and AXIN2 mutated samples. c Kaplan Meier of the overall survival of patients with AXIN2 mutation. d Mutational profile of the most mutated genes in the AXIN2 mutated patients.
alteration of other genes related with the WNT pathway. Nonetheless, the most frequently activated signaling in metasta
cic CRC is the WNT pathway. For this reason, we performed
additional in silico analysis. This analysis showed that mutations in AXIN2 found in CRC patients were more frequently in earlier
stages of tumor samples derived from the right colon than those
derived from the left. Furthermore, patients who carry mutations in the AXIN2 gene have a worse prognosis \((p < 0.05)\). This fact,
remark the importance to identify biomarkers for CRC in
population, such as AXIN2 variations in patients with Hypodontia.
However, one limitation of our study is the fact that we could
not document hypodontia in patients with CRC because we did
not have history records of these individuals. Then, we could not
establish if the cause of tooth absence was extraction or
hypodontia.

Our study showed a statistical significant difference between
familial cancer history in individuals with hypodontia and AXIN2
rs2240308 variations \((p < 0.005 \ OR = 1.75, \ 95\% \ CI: 1.22-6.91)\). Gastric cancer was the most frequent cancer in this association.
The association between AXIN2 and gastric cancer has been
reported,\(^{34}\) but the association between hypodontia and gastric
cancer has not been demonstrated.\(^{15,35}\) Predisposition to CRC is
not have history records of these individuals. Then, we could not
document hypodontia in patients with CRC because we did
did not have history records of these individuals. Then, we could not
establish if the cause of tooth absence was extraction or
hypodontia.

According to the results from the present research, upper lateral
incisor was the most frequent missing teeth among patients with the
AXIN2 rs2240308, although these associations were not
statistically significant. In contrast, AXIN2 rs2240308 showed
association with hypodontia in individuals from Brazil who had
at least one missing tooth, although one conclusion of this study
was that dental agenesis arises from multiple AXIN2 gene
variants.\(^{37}\) Mutations in AXIN2 have been mainly associated with
moderate oligodontia which affects incisors and also individuals
with severe oligodontia which is caused by two truncated AXIN2
proteins.\(^{4,58}\) In conclusion, the results of the present study showed an
association between AXIN2 rs2240308 and CRC in Colombian
population. In silico analysis demonstrated that patients who carry
any mutations of AXIN2 have worse prognosis. Future studies
should be performed to identify biomarkers in CRC and its
association with hypodontia and to identify target molecules for
CRC treatment.

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