Original Paper

Assessment of the KRAS rs61764370 Polymorphism as a Genetic Risk Factor for Colorectal Cancer in a Romanian Population-A Preliminary Study

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ABSTRACT: Background: The KRAS 3′UTR let-7 complementary site 6 (LCS6) polymorphism rs61764370 has been proposed as a susceptibility marker for colorectal cancer (CRC), but most published work has addressed prognosis and treatment response rather than disease risk, and no case-control data exist for Eastern European populations. Methods: We genotyped rs61764370 by TaqMan allelic discrimination in 107 patients with histologically confirmed colorectal adenocarcinoma and 329 cancer-free controls from South-West Romania. Susceptibility was assessed under codominant, dominant, recessive, and allelic models. Results: Genotype distributions were consistent with Hardy-Weinberg equilibrium in both cases (p=0.596) and controls (p=1.000). The G allele frequency was 10.3% in cases and 7.0% in controls. The TG genotype was more frequent in cases (20.6%) than controls (13.4%), but no comparison reached significance: codominant OR=1.671 (p=0.074), dominant OR=1.633 (p=0.086), and allelic OR=1.524 (p=0.119). The recessive model could not be reliably estimated, as no GG homozygote was found among cases and only one among controls. Genotype distribution was not significantly associated with sex, tumor location, stage, or histological grade. Conclusions: rs61764370 was not significantly associated with colorectal cancer susceptibility in this Romanian population. Estimates were consistently above unity but imprecise, and the near absence of GG homozygotes precluded evaluation of recessive inheritance. Larger studies are required before a modest susceptibility effect can be excluded.

KEYWORDS: *Colorectal cancer risk, KRAS 3'UTR, rs61764370, let-7 binding site, Eastern European population.*

Introduction

Colorectal cancer remains one of the most diagnosed malignancies and a leading cause of cancer-related death worldwide, ranking third in incidence and second in mortality according to the most recent GLOBOCAN estimates [1,2].

The global burden is projected to rise further, driven largely by population aging and the increasing adoption of Westernized dietary and lifestyle patterns [3].

Within Europe, incidence and mortality vary considerably by region, and Romania continues to report one of the highest colorectal cancer mortality rates among European Union member states [2,4], underscoring the need for region-specific research into the genetic determinants of disease risk in this population.

Colorectal carcinogenesis is a multistep process driven by the sequential accumulation of genetic and epigenetic alterations affecting oncogenes and tumor suppressor genes, among which mutations in the KRAS oncogene are among the most frequent and best characterized [5,6].

Beyond somatic mutations, germline variation in regulatory, non-coding regions of KRAS has attracted growing interest as a potential modifier of individual cancer susceptibility [7,8].

MicroRNAs are short, evolutionarily conserved non-coding RNAs that regulate gene expression post-transcriptionally by binding to

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complementary sequences within the 3′ untranslated regions of target messenger RNAs, leading to translational repression or transcript degradation [9,10].

The let-7 family, first described as a regulator of developmental timing in Caenorhabditis elegans [11,12], acts as a tumor suppressor in humans by restraining genes involved in cell cycle progression and proliferation, and reduced let-7 activity has been linked to aggressive behavior across multiple tumor types, including colorectal cancer [13,14].

The KRAS 3′ untranslated region contains a let-7 complementary site, and a common polymorphism located within it, rs61764370 (T>G), was initially shown to weaken let-7 binding, with proposed downstream consequences for KRAS regulation [8,13].

Because increased KRAS expression is mechanistically plausible as a driver of both tumor initiation and more aggressive tumor biology, the variant has been proposed as a candidate biomarker of both cancer risk and tumor behavior.

It was first associated with increased risk of non-small cell lung cancer among moderate smokers [8], and subsequently with poor outcome and platinum resistance in ovarian cancer [7].

However, studies in colorectal cancer have produced inconsistent results, and most have addressed prognosis and treatment response rather than susceptibility: improved outcome with salvage anti-epidermal growth factor receptor monotherapy [15] and improved survival in early-stage disease have been reported [16], whereas other series found no effect on response or survival in metastatic disease [17], and a dedicated meta-analysis found no consistent prognostic effect [18].

This distinction matters, since a variant may influence disease risk, tumor behavior, or treatment response through partially independent mechanisms.

Pooled analyses in Caucasian populations have found no significant association between rs61764370 and colorectal cancer risk [19,20].

However, the primary case-control studies contributing to these syntheses were drawn almost exclusively from Northern, Western, and Central European and North American cohorts, and no data from South-Eastern Europe have been available for inclusion.

Evidence from other digestive malignancies illustrates the value of population-specific designs: the miR-149 rs2292832 polymorphism showed no association with gastric cancer susceptibility in a Romanian cohort, despite conflicting reports from Asian populations [21].

MicroRNA-pathway polymorphisms frequently show ethnicity-dependent associations with cancer risk [21,22].

Systematic evaluation of microRNA-pathway polymorphisms in colorectal cancer has shown that significant associations are frequently confined to specific populations and genetic models, with several loci showing opposing effects in East Asian and European cohorts [23].

Conversely, a recent Romanian study identified a significant association between the GAS5 rs145204276 insertion/deletion polymorphism and colorectal cancer risk [24].

In the largest systematic screen of microRNA-binding-site variants conducted in a European population, rs61764370 was among the loci showing no association with disease risk under any genetic model [25].

Taken together, these observations argue against extrapolating susceptibility data across ethnic groups and support the need for population-specific analyses of candidate microRNA-pathway variants in the Romanian population.

Objective

The present study aimed to investigate whether the KRAS rs61764370 T>G polymorphism is associated with colorectal adenocarcinoma susceptibility in a Romanian case-control population.

To address this hypothesis, we compared KRAS rs61764370 genotype and allele frequencies between histologically confirmed colorectal adenocarcinoma cases and cancer-free controls from South-West Romania, evaluating the association under codominant, dominant, recessive, and allelic genetic models.

As a secondary exploratory aim, we assessed whether the distribution of rs61764370 differed according to sex, tumor location, stage at diagnosis, and histological grade among colorectal cancer cases.

The analysis is of particular interest because rs61764370 shows substantial geographic variation in allele frequency, while susceptibility data from South-Eastern Europe remains scarce.

Population-specific estimates are therefore valuable both for the interpretation of pooled evidence and for the design of future studies in this region.

The study was not designed to assess prognosis or treatment response, and the results should not be extrapolated to these outcomes.

Methods

Subjects

This hospital-based case-control study included patients with histologically confirmed colorectal adenocarcinoma and unrelated, cancer-free control subjects, recruited at the Human Genomics Laboratory of the University of Medicine and Pharmacy of Craiova and the Sf. Nectarie Oncology Center, Craiova, Romania.

Cases were eligible when the diagnosis had been established by histopathological examination of biopsy or surgical specimens.

Controls were recruited during the same period from individuals hospitalized for non-neoplastic conditions; those with a personal history of malignancy or known digestive tract disease were excluded.

Controls were selected to approximate the age and sex distribution of cases.

The final analysis included 107 cases and 329 controls.

Demographic and clinicopathological information were extracted from medical records and included sex, primary tumor location (colon or rectum), pathological stage according to the American Joint Committee on Cancer (AJCC) staging system, and histological grade.

DNA extraction and genotyping

Peripheral blood was collected into EDTA tubes. Genomic DNA was isolated from peripheral blood leukocytes using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA), according to the manufacturer's protocol.

DNA concentration and purity were assessed spectrophotometrically, and samples were diluted to a working concentration of 10-20ng/µL with a minimum 260/280 absorbance ratio of 1.7.

Genotyping was performed with a TaqMan SNP Genotyping Assay (Applied Biosystems, Waltham, MA, USA; assay identifier C_89129087_10) in 5µL reactions containing 2.5µL TaqMan Genotyping Master Mix, 0.25µL 20x assay mix, 1.25µL genomic DNA and 1µL nuclease-free water.

Amplification was carried out in 384-well optical plates on a ViiA7 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA), with an initial denaturation at 95°C for 10min followed by 40 cycles of 95°C for 15 s and 60°C for 1min, with fluorescence acquisition during the annealing and extension step.

Genotypes were assigned by allelic discrimination using QuantStudio Software v1.3 (Thermo Fisher Scientific, Waltham, MA, USA) (Figure 1).

No-template controls (NTC) were included in every run, and genotyping was performed blinded to case-control status.

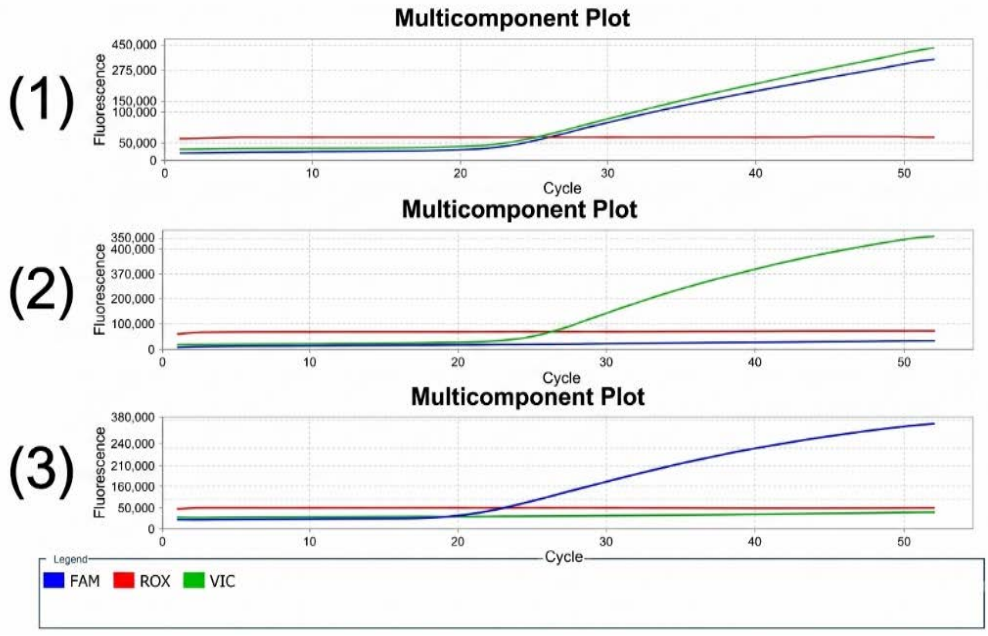


Figure 1. Allelic discrimination according to FAM and VIC for rs61764370 T>G SNP-(1)TG, (2)TT, (3)GG genotypes, while fluorophore ROX is used for normalization; Fluorophore FAM corresponds to wavelengths 494nm-518nm, fluorophore ROX corresponds to wavelengths 567nm-591nm, fluorophore VIC corresponds to wavelengths 538nm-554nm.

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Statistical analysis

Genotype and allele frequencies were calculated by direct counting.

Differences between cases and controls were assessed using Pearson's chi-square test, with Fisher's exact test where expected cell counts were small.

Hardy-Weinberg equilibrium was evaluated separately in cases and controls using an exact test because of the low expected frequency of the variant homozygous genotype.

Association with susceptibility was evaluated under codominant, dominant, recessive, and allelic models, with odds ratios and 95% confidence intervals calculated from two-by-two contingency tables.

Because the GG genotype was exceptionally rare, the recessive and GG-specific contrasts were interpreted cautiously.

Exploratory case-only analyses examined genotype distribution according to sex, tumor location, stage, and histological grade.

Two-sided p values below 0.05 were considered statistically significant.

Results

The final analysis included 436 participants, comprising 107 patients with histologically confirmed colorectal adenocarcinoma and 329 cancer-free controls.

A valid KRAS rs61764370 genotype was available for all participants.

Overall, the TT genotype was identified in 369 individuals (84.6%), the TG genotype in 66 (15.1%), and the GG genotype in one participant (0.2%).

Among patients with colorectal cancer, 85 of 107 individuals had the TT genotype (79.4%) and 22 had the TG genotype (20.6%); no patient carried the GG genotype.

Among controls, the TT, TG, and GG genotypes were observed in 284 of 329 (86.3%), 44 of 329 (13.4%), and one of 329 participants (0.3%), respectively.

The overall genotype distribution did not differ significantly between cases and controls (p=0.134) (Table 1).

Table 1. Distribution of KRAS rs61764370 genotypes in colorectal cancer cases and controls.

Study group	Genotype TT, n (%)	Genotype TG, n (%)	Genotype GG, n (%)	Total, n (%)
CRC cases	85 (79.4%)	22 (20.6%)	0 (0.0%)	107 (100%)
Controls	284 (86.3%)	44 (13.4%)	1 (0.3%)	329 (100%)
Overall	369 (84.6%)	66 (15.1%)	1 (0.2%)	436 (100%)

Allele frequencies and Hardy-Weinberg equilibrium

Among cases, the T allele was observed 192 times, corresponding to a frequency of 89.7%, whereas the G allele was observed 22 times, corresponding to a frequency of 10.3%.

Among controls, the T and G allele frequencies were 93.0% and 7.0%, respectively.

Genotype distributions were consistent with Hardy-Weinberg equilibrium in both cases (p=0.596) and controls (p=1.000). (Table 2)

Table 2. Allele frequencies and Hardy-Weinberg equilibrium testing for KRAS rs61764370 in cases and controls.

	Observed TT	Observed TG	Observed GG	T allele (%)	Gallele (%)	HWE p
CRC cases	85	22	0	89.7	10.3	0.596
Controls	284	44	1	93.0	7.0	1.000

Association between KRAS rs61764370 and colorectal cancer susceptibility

Under the codominant model, the TG genotype was more frequent among cases than controls, occurring in 20.6% and 13.4% of participants, respectively.

Compared with the TT genotype, TG was associated with higher estimated odds of colorectal cancer, although the association did not reach statistical significance (OR=1.671, 95% CI: 0.948-2.943; p=0.074) (Table 3).

A similar pattern was observed under the dominant model. Carriers of at least one G allele had higher estimated odds of colorectal cancer than TT homozygotes, but the result remained below the conventional threshold for statistical significance (OR=1.633, 95% CI: 0.929-2.873; p=0.086).

In the allelic analysis, the G allele was more frequent among cases than controls, 10.3% versus 7.0%.

Nevertheless, the difference was not statistically significant (OR=1.524, 95% CI: 0.894-2.599; p=0.119).

The recessive model could not be estimated reliably because no GG homozygotes were identified among cases and only one was observed among controls.

Table 3. Association between KRAS rs61764370 and colorectal cancer susceptibility under codominant, dominant, allelic and recessive genetic models.

Genetic model	Comparison	CRC Cases	Controls	OR*	95% CI	p
Codominant	TG versus TT	22/85	44/284	1.671	0.948-2.943	0.074
Dominant	TG+GG versus TT	22/85	45/284	1.633	0.929-2.873	0.086
Allelic	G versus T	22/192	46/612	1.524	0.894-2.599	0.119
Recessive	GG versus TT+TG	0/107	1/328	-	-	-

**OR-odds ratio; CI-confidence interval.*

Clinicopathological characteristics of the colorectal cancer group

The CRC case group included 51 women (47.7%) and 56 men (52.3%).

The primary tumor was located in the colon in 57 patients (53.3%) and in the rectum in 50 patients (46.7%).

Five patients had stage I disease (4.7%), 26 had stage II disease (24.3%), 47 had stage III disease (43.9%), and 29 had stage IV disease (27.1%).

When grouped into broader clinical categories, 31 patients (29.0%) had early-stage disease, defined as stages I-II, whereas 76 patients (71.0%) had advanced disease, defined as stages III-IV.

Regarding histological differentiation, 38 tumors were classified as grade 1 (35.5%), 52 as grade 2 (48.6%), and 17 as grade 3 (15.9%) (Table 4).

Table 4. Clinicopathological characteristics of the colorectal cancer group.

		N (%)
Sex	Female	51 (47.7%)
	Male	56 (52.3%)
Tumor location	Colon	57 (53.3%)
	Rectum	50 (46.7%)
Stage at diagnosis	I	5 (4.7%)
	II	26 (24.3%)
	III	47 (43.9%)
	IV	29 (27.1%)
Grouped stage	I-II	31 (29.0%)
	III-IV	76 (71.0%)
Histological grade	Grade 1	38 (35.5%)
	Grade 2	52 (48.6%)
	Grade 3	17 (15.9%)

Association between genotype and clinicopathological characteristics

Genotype distribution according to clinicopathological characteristics is presented in Table 5.

Among women, the TT genotype was identified in 41 of 51 patients (80.4%), whereas 10 women carried the TG genotype (19.6%).

Among men, 44 of 56 patients had the TT genotype (78.6%) and 12 had the TG genotype (21.4%).

Compared with women, men had similar odds of carrying the TG genotype (OR=1.118, 95% CI: 0.436-2.865; p=0.816).

Among patients with rectal tumors, 40 of 50 had the TT genotype (80.0%) and 10 had the TG genotype (20.0%).

Among patients with colon tumors, the TT and TG genotypes were found in 45 of 57 (78.9%) and 12 of 57 patients (21.1%), respectively.

Patients with colon tumors had similar odds of carrying the TG genotype to those with rectal tumors (OR=1.067, 95% CI: 0.416-2.734; p=0.893).

The TG genotype was identified in none of the five patients with stage I disease, in 6 of 26 patients with stage II disease (23.1%), in 8 of 47 patients with stage III disease (17.0%), and in 8 of 29 patients with stage IV disease (27.6%).

The overall genotype distribution did not differ significantly across the four stage categories (χ^2=2.632, p=0.452).

When stages were grouped, the TG genotype was present in 6 of 31 patients with stage I-II disease (19.4%) and in 16 of 76 patients with stage III-IV disease (21.1%).

Advanced-stage disease was not associated with TG carriage (OR=1.111, 95% CI: 0.390-3.168; p=0.844).

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The TG genotype was observed in 10 of 38 patients with grade 1 tumors (26.3%), 11 of 52 patients with grade 2 tumors (21.2%), and 1 of 17 patients with grade 3 tumors (5.9%).

Although TG carriage was numerically less frequent in poorly differentiated tumors, the overall difference across the three grade categories was not statistically significant (χ^2=3.024, p=0.220).

The low number of patients in several categories, particularly stage I and TG-positive grade 3 disease, limits the interpretation of these numerical differences (Table 6).

After grouping grades 2 and 3, patients with grade 2-3 tumors had lower estimated odds of carrying the TG genotype than patients with grade 1 tumors (OR=0.589, 95% CI: 0.227-1.529; p=0.274).

Table 5. KRAS rs61764370 genotype distribution according to clinicopathological characteristics (case-only analysis).

		TT, n (%)	TG, n (%)	OR	95% CI	p
Sex	Female	41 (80.4)	10 (19.6)	Reference	-	-
	Male	44 (78.6)	12 (21.4)	1.118	0.436-2.865	0.816
Tumor location	Rectum	40 (80.0)	10 (20.0)	Reference	-	-
	Colon	45 (78.9)	12 (21.1)	1.067	0.416-2.734	0.893
Grouped stage	I-II	25 (80.6)	6 (19.4)	Reference	-	-
	III-IV	60 (78.9)	16 (21.1)	1.111	0.390-3.168	0.844
Histological grade	Grade 1	28 (73.7)	10 (26.3)	Reference	-	-
	Grades 2-3	57 (82.6)	12 (17.4)	0.589	0.227-1.529	0.274

Table 6. KRAS rs61764370 genotype distribution across individual categories of stage at diagnosis and histological grade.

		Total, n	TT, n (%)	TG, n (%)	Global p
Stage at diagnosis	I	5	5 (100.0)	0 (0.0)	0.452
	II	26	20 (76.9)	6 (23.1)	
	III	47	39 (83.0)	8 (17.0)	
	IV	29	21 (72.4)	8 (27.6)	
Histological grade	Grade 1	38	28 (73.7)	10 (26.3)	0.220
	Grade 2	52	41 (78.8)	11 (21.2)	
	Grade 3	17	16 (94.1)	1 (5.9)	

Discussion

This case-control study tested whether the KRAS rs61764370 polymorphism is associated with colorectal adenocarcinoma susceptibility in a population from South-West Romania.

The hypothesis was not confirmed: no statistically significant association emerged under any genetic model.

Odds ratios were directionally consistent, above unity in the codominant, dominant, and allelic comparisons, but these derive largely from the same underlying counts and do not constitute independent confirmation.

The confidence intervals make a large effect unlikely while remaining compatible both with no effect and with a modest increase in susceptibility.

The result is therefore best read as an absence of evidence at the achievable resolution rather than as evidence of absence.

Our point estimates are higher than those reported in the largest comparable European series, although the confidence intervals overlap substantially.

Schneiderova et al. genotyped 1,080 Czech colorectal adenocarcinoma cases and 1,442 controls and found no association under any genetic model, with a dominant-model odds ratio of 1.05 (95% CI: 0.81-1.37); their estimates were adjusted for sex, age, smoking, body mass index, family history, education, and area of residence, and with no substantial change in the results [25].

Kjersem et al. compared 197 colorectal cancer patients, 1,060 individuals with colorectal polyps, and 358 healthy controls from a Norwegian screening cohort, finding carrier frequencies of 23%, 20%, and 20% respectively [17].

Uvirova et al. detected the heterozygous genotype in 12.4% of 428 Czech colorectal tumors, a frequency not significantly different from that observed in breast, lung, or brain tumors [26].

The divergence from the Czech estimate is instructive: carrier frequency among our cases

(20.6%) exceeds theirs (15.9%), while among our controls (13.4%) it falls slightly below theirs (15.8%).

Each difference is modest and within the range expected from sampling variation at our sample size, yet their coincidence in opposite directions suffices to generate the observed odds ratio.

The pooled results consistently indicate no association. In a meta-analysis restricted to Caucasian populations comprising 30 studies, 14,936 cases and 15,168 controls, no association was found between the variant genotype and cancer overall (OR=1.05, 95% CI: 0.97-1.14) or within the colorectal subgroup (OR=1.13, 95% CI: 0.84-1.54) [20].

An analysis of 29 studies with 14,796 cases and 14,985 controls reached an equivalent conclusion [19], and a systematic review of 85 studies covering 21 microRNA-binding-site polymorphisms again found rs61764370 among the variants without significant association with colorectal cancer risk in any genetic model [23].

Our confidence intervals contain the pooled colorectal estimate of 1.13 in all three genetic models, and the present data are therefore in accordance with the aggregated literature despite higher point estimates.

The variant allele shows pronounced geographic structure, which is directly relevant to interpretation.

Among the 2,433 samples across 46 geographic populations, the minor allele frequency was 5.8% overall and approximately 7.6% among individuals of European descent [8].

Reported control-group frequencies in European series cluster within a narrow band, at approximately 8% in Czech controls [25] and approximately 10% in Norwegian screening controls [17].

In our control group, the G allele frequency was 7.0%, placing the South-West Romanian population at the lower end of, but within, the range described for other European groups.

By contrast, the variant is almost absent from East Asian populations, where its frequency is below 1% [20].

One methodological caution deserves emphasis: the literature reports variously carrier frequencies, heterozygote genotype frequencies, and allele frequencies, and these are not interchangeable, since a carrier prevalence of 20% corresponds to an allele frequency of approximately 10% [17].

The frequencies given here are allele frequencies throughout.

The discrepancy between the original functional characterization of the let-7 complementary site and consistent lack of evidence for an association with susceptibility merits comment, because subsequent evidence suggests the mechanistic premise is weaker than initially supposed.

Targeted knock-in of rs61764370 was shown not to alter KRAS expression, although it did reduce let-7 levels [27], indicating a more restricted biological effect than originally proposed.

Bioinformatic and expression analyses point in the same direction: no significant expression quantitative trait locus for rs61764370 and KRAS was identified in any tissue, the site is not evolutionarily conserved, and thermodynamic modelling predicted stronger, not weaker, binding in the presence of the variant allele [25].

Functional redundancy offers a further explanation, since 10 let-7 complementary sites exist within the KRAS 3′ untranslated region and binding at the remaining 9 may buffer net KRAS output [20].

Tissue-specific differences in let-7 abundance and KRAS dependency may also explain why associations reported in ovarian, breast, and lung cancer are not reproduced in colorectal cancer.

The distinction between susceptibility and clinical outcome is an important consideration when interpreting the available literature.

A germline variant may have little effect on the initial development of colorectal cancer while still influencing tumor biology or treatment sensitivity after diagnosis, and evidence from studies of anti-epidermal growth factor receptor therapy or survival should not be read as evidence of an effect on disease risk.

Most published work on rs61764370 in colorectal cancer concerns the latter aspect and is directionally inconsistent: improved outcome with salvage anti-epidermal growth factor receptor monotherapy [15], improved survival in early-stage disease [16], and variable effects according to the chemotherapy backbone [28] have all been reported.

The meta-analysis by Langevin and Christensen, covering six studies and 1,672 patients, found no association between genotype and overall survival (HR=0.86, 95% CI: 0.62-1.19) or progression-free survival (HR=1.06, 95% CI: 0.79-1.42) [18].

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The present study addresses susceptibility only, and neither supports nor contradicts these prognostic reports.

Within the case group, genotype was not associated with sex, tumor location, stage, or histological grade.

Carrier frequency decreased from 26.3% in grade 1 tumors to 21.2% in grade 2 and 5.9% in grade 3; however, only one grade 3 patient carried the variant genotype, and neither the overall nor the grouped comparison approached significance.

The apparent gradient is most plausibly attributed to random variation in a small subgroup and should not be interpreted as a protective effect.

These comparisons are descriptive: a case-only design cannot address susceptibility, and stage and grade describe the extent and behavior of established disease rather than determinants of its occurrence.

Limitations

Several limitations should be acknowledged.

The modest number of cases and the low frequency of the variant allele limited the precision of the susceptibility estimates, particularly for small or moderate effects.

The GG genotype was observed in only one participant, precluding evaluation of a recessive model; this constraint is intrinsic to the locus, since the homozygous variant genotype was present in 10 of 1,425 Czech controls (0.7%) [25] and in none of 428 Czech colorectal tumors [26], so approximately two homozygous controls and one homozygous case would be expected in a cohort of our size.

The susceptibility estimates are unadjusted, and residual confounding by smoking, body mass index, or family history cannot be excluded, although the fully adjusted analysis of Schneiderova et al. in a cohort ten times larger was likewise negative [25].

The study was hospital-based, which may limit generalizability; the case-only analyses were based on small subgroups; and a single polymorphism was evaluated without functional validation.

Strengths

The study contributes population-specific data from an Eastern European cohort in which rs61764370 has not previously been characterized, addressing a geographic gap in a body of evidence dominated by Northern and Western European, Czech, and North American series.

Conclusions

The KRAS rs61764370 polymorphism was not significantly associated with colorectal cancer susceptibility in this Romanian population, and showed no relationship with sex, tumor location, stage, or histological grade.

The variant allele frequency of 7.0% among controls places this population within the European range.

A modest susceptibility effect cannot be excluded at the present sample size.

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None to declare.

Author Contributions

Conceptualization, T.C.B., F.B., M.G.C., M.-L.C. and M.S.; methodology, T.C.B., F.B., M.G.C. and M.S.; software, M.-L.C. and T.C.B.; validation, All authors; formal analysis T.C.B., M.G.C. and M.-L.C.; investigation, T.C.B., M.G.C., S.S.S., B.C.A., F.B., M.-L.C. and M.S.; resources, T.C.B., M.G.C., F.B. and M.S.; data curation, T.C.B., P.O.S.; writing-original draft preparation, T.C.B., F.B., M.G.C., M.-L.C. and M.S.; writing-review and editing, F.B., M.G.C., M.-L.C. and M.S.; visualization, S.S.S., A.-M.C., A.M.M., P.O.S., R.A.S., B.C.A.; supervision M.S., F.B. and M.-L.C.; project administration, M.S.

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Conflicts of interest

The authors declare no competing interests.

Institutional Review Board

This study was conducted in accordance with the Declaration of Helsinki, first established in 1964 and revised in 2024.

It received approval from the Ethics Committee of the University of Medicine and Pharmacy of Craiova nr. 245/25.10.2023, date of approval 25 October 2023.

Consent Statement

All human subjects involved in this study provided a written informed consent prior to participation, including the consent of publishing their anonymized data.

Data availability

All data presented in the manuscript are available from the authors upon request.

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