Submitted: 30 June, 2025 Accepted: 27 August, 2025 Published: 15 December, 2025 DOI:10.22514/ejgo.2025.142

ORIGINAL RESEARCH

European Journal of Gynaecological Oncology

KRAS exon 2 mutations in mucinous ovarian carcinoma: an exploratory study of low prevalence and grade association in Azerbaijan

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Abstract

Background: Ovarian cancer represents a leading cause of global morbidity and mortality among gynecological malignancies. Kirsten rat sarcoma (KRAS) gene mutations, particularly in Exon 2, play a significant role in the development of mucinous ovarian carcinoma (MOC); however, studies investigating this mutation in specific populations remain limited. This exploratory study aimed to investigate the prevalence of KRAS Exon 2 mutations and their clinicopathological correlates in MOC patients from Azerbaijan. **Methods**: This cross-sectional exploratory study involved 25 patients with mucinous ovarian carcinoma who had available paraffin blocks and met the inclusion criteria. KRAS mutations were detected using polymerase chain reaction (PCR) and sequencing techniques. Comprehensive clinicopathological data, including bilaterality, age, cancer stage, histological grading, growth pattern, and tumor markers, were systematically analyzed using the Chi-square test and Fisher's exact test for categorical variables, and *t*-tests or nonparametric equivalents for continuous variables. **Results**: A total of 25 participants met the inclusion criteria. KRAS Exon 2 mutations were detected in 12% of patients. Power analysis revealed that the current sample size could detect large effect sizes (Cohen's $w \geq 0.55$) with 80% power. No significant associations were found between bilaterality ($p = 0.565$), age ($p = 0.089$), cancer stage ($p = 0.518$), and KRAS mutations. However, a statistically significant association was observed between histological grading and KRAS mutations ($p = 0.038$), specifically with Grade 3 tumors showing a higher mutation frequency. Mutations were more frequent in patients with an expansile growth pattern (67% *vs.* 33%) and elevated tumor markers, though these associations did not reach statistical significance. **Conclusions**: This exploratory study demonstrates that KRAS Exon 2 mutations are significantly associated with a higher histological grade in MOC, suggesting their potential role as prognostic biomarkers and therapeutic targets. The 12% mutation rate is lower than global averages, indicating possible population-specific genetic variations. Larger multicenter studies are needed to validate these findings.

Keywords

Mucinous ovarian carcinoma; KRAS mutations; Exon 2; Histological grading; Prognostic biomarker; Azerbaijan

1. Introduction

Ovarian cancer remains a significant global health challenge, ranking among the leading causes of cancer-related morbidity and mortality in women worldwide. Within the spectrum of epithelial ovarian cancers (EOC), mucinous ovarian carcinoma (MOC) represents a distinct and relatively rare subtype, accounting for approximately 3–5% of all EOC cases [1]. Despite its rarity, research on MOC is particularly important due to its distinct clinical behavior and noted resistance to conventional therapies, making the understanding of its molecular landscape crucial for developing targeted treatments. Unlike the more common high-grade serous ovarian carcinoma (HGSOC), MOC presents unique clinical and molecular characteristics. It frequently affects younger women, often under the age of 40, and is typically diagnosed at an early stage (International Federation of Gynecology and Obstetrics-FIGO stage I–II in 70–80% of cases) [1, 2]. Histopathologically, MOC is characterized by gastrointestinal-type or endocervical-like epithelium and is further classified into expansile and infiltrative growth patterns, which carry different prognostic implications. The expansile subtype generally demonstrates a better prognosis compared to the infiltrative subtype, with the latter showing more aggressive behavior and poorer outcomes

[1].

Molecularly, MOC diverges significantly from HGSOC. While HGSOC is often characterized by widespread genomic instability and frequent tumor protein p53 (*TP53*) mutations, MOC commonly harbors mutations in the Kirsten rat sarcoma viral oncogene homolog (KRAS) gene, alongside potential amplifications in human epidermal growth factor receptor 2 (HER2). KRAS mutations, particularly activating mutations within Exon 2 (such as codons G12 and G13), are considered key drivers in the pathogenesis of MOC, occurring in a substantial proportion of cases [3]. Reported frequencies range widely from 40% to over 85% depending on the study cohort and histological subtype [1, 2]. This wide range can be attributed to differences in study cohorts, geographical variations, and methodologies used for mutation detection [4]. These mutations constitutively activate the Rat sarcoma virus/mitogen-activated protein kinase (*RAS/MAPK*) signaling pathway, promoting uncontrolled cell proliferation, survival, and growth [5]. Importantly, the prevalence of KRAS mutations appears to be higher in the expansile subtype compared to the infiltrative subtype of MOC, which may partially explain the prognostic differences between these subtypes.

The distinct molecular profile of MOC, dominated by KRAS mutations, contributes to its unique clinical behavior, including a notable resistance to conventional platinum-based chemotherapy regimens that are standard for other EOC types [1, 6]. This chemoresistance underscores the need for alternative, targeted therapeutic strategies. The presence of KRAS mutations, therefore, holds significant potential not only for understanding MOC tumorigenesis but also as a biomarker for prognosis and guiding personalized treatment approaches, potentially involving mitogen-activated protein kinase enzymes (*MEK*) inhibitors or novel direct KRAS inhibitors [1, 2].

Despite the recognized importance of KRAS mutations in MOC development and progression, comprehensive studies correlating these mutations with specific clinicopathological features and patient outcomes remain relatively limited, particularly within specific geographic populations. Understanding these correlations within diverse populations is crucial for refining diagnostic and therapeutic strategies globally [7]. Reported KRAS mutation frequencies in MOC show geographical variation, ranging from 29% in Malaysian populations to 92% in Western cohorts [2, 4, 8]. This geographical variation suggests potential ethnic or population-specific genetic influences on KRAS mutation prevalence, highlighting the importance of regional studies. The Caucasus region, encompassing countries such as Azerbaijan, Georgia, and Armenia, represents an understudied population in terms of MOC molecular characteristics. This region's unique genetic background, influenced by both European and Asian ancestries, may contribute to distinct patterns of KRAS mutations in MOC. Understanding the molecular landscape of MOC in this population is essential for developing region-specific diagnostic and therapeutic approaches.

Therefore, this exploratory study aimed to investigate the frequency of KRAS Exon 2 mutations in a cohort of patients with mucinous ovarian carcinoma from Azerbaijan and to analyze the relationship between these mutations and various clinicopathological parameters, including tumor bilaterality, patient age, cancer stage, histological grading, growth pattern, tumor location, and tumor marker levels. By elucidating these relationships, we sought to contribute to the understanding of MOC characteristics in this specific population and provide insights into the potential prognostic and therapeutic implications of KRAS mutations in this understudied region.

2. Methods

2.1 Study design and patient cohort

This study employed a single-center cross-sectional exploratory design to investigate the association between KRAS Exon 2 mutations and clinicopathological features in patients diagnosed with mucinous ovarian carcinoma (MOC). The exploratory nature of this study is emphasized due to the limited sample size and the need for cautious interpretation of results. The study cohort comprised 25 patients who received a diagnosis and treatment within the Department of Oncology, Azerbaijan Medical University, between January 2020 and December 2024. This 5-year period represents the maximum available timeframe for patient recruitment at our institution, reflecting the rarity of MOC, which accounts for only 3–5% of all epithelial ovarian cancers. Inclusion criteria mandated a confirmed histopathological diagnosis of MOC, complete clinical records, and the availability of formalin-fixed, paraffin-embedded (FFPE) tissue blocks suitable for molecular analysis. Patients were included after meeting all predefined inclusion criteria, ensuring the cohort was representative of the study's objectives.

This retrospective study received ethical approval from the Clinical Research Ethics Committee of Azerbaijan Medical University (Decision number #238, dated 06 December 2023). Due to the retrospective and observational nature of the study, which involved the analysis of archived tissue samples and medical records without any intervention or contact with patients, the ethics committee waived the requirement for written informed consent from participants. All patient data were anonymized and handled in accordance with the institutional guidelines and international ethical standards for medical research.

Given the exploratory nature of this study and the rarity of MOC, a *post-hoc* power analysis was performed using G*Power 3.1.9.7 software (Heinrich Heine University Düsseldorf, Düsseldorf, NRW, Germany) to determine the detectable effect sizes with the current sample size. With a sample size of 25 patients, an alpha level of 0.05, and desired power of 0.80, the study could detect large effect sizes (Cohen's $w \geq 0.55$) for categorical associations. For detecting medium effect sizes (Cohen's $w = 0.3$), approximately 87 patients would be required. This limitation necessitates cautious interpretation of negative results, as the study may be underpowered to detect associations with small to moderate effect sizes. The risk of Type II error (false negative results) is acknowledged, particularly for associations with age, tumor markers, and other clinical parameters that may require larger sample sizes to detect meaningful relationships. Future multi-center collaborative studies are recommended to achieve adequate statistical

power for comprehensive analysis.

Comprehensive clinicopathological data were retrospectively collected for all included patients from institutional medical records and pathology reports. The variables extracted included patient age at diagnosis, tumor bilaterality (unilateral or bilateral involvement), International Federation of Gynecology and Obstetrics (FIGO) cancer stage at diagnosis, histological grade of the tumor (*e.g.*, Grade 1, 2, or 3), and pre-treatment levels of tumor markers (*e.g.*, CA125, CEA). Histological grading was performed according to established criteria, with Grade 1 representing well-differentiated tumors, Grade 2 moderately differentiated tumors, and Grade 3 poorly differentiated tumors. Growth patterns were classified as expansile (characterized by smooth, pushing borders) or infiltrative (characterized by irregular, invasive borders with stromal infiltration). Tumor marker levels were categorized as elevated or normal based on established reference ranges (CA125 >35 U/mL, CEA >5 ng/mL).

Data collection was performed meticulously by trained personnel to ensure accuracy and completeness for subsequent analysis. All data were double-checked against the original medical records to minimize transcription errors.

Genomic DNA was extracted from the FFPE tumor tissue samples using QIAamp DNA FFPE Kit (Qiagen #56404, Qiagen, Hilden, NRW, Germany) following the manufacturer's protocols, optimized for archival tissues. The presence of mutations within Exon 2 of the KRAS gene was assessed using a combination of Polymerase Chain Reaction (PCR) and direct Sanger sequencing. Specific primers flanking KRAS Exon 2 (F: 5′-GGCCTGCTGAAAATGACTGA-3′, R: 5′-GTCCTGCACCAGTAATATGC-3′) were utilized for PCR amplification. Following amplification, the PCR products were purified and subjected to bidirectional Sanger sequencing (ABI 3730xl Thermo Fisher Genetic Analyzer, Thermo Fisher Scientific, Waltham, MA, USA) to identify any nucleotide variations compared to the reference KRAS sequence (NM_004985.5). All identified mutations were confirmed through repeat analysis Figs. 1,2,3.

To ensure the reliability of our mutation detection methodology and exclude technical factors as a cause of low mutation frequency, several quality control measures were implemented. DNA quality and quantity were assessed using spectrophotometry (NanoDrop 2000), with only samples showing A260/A280 ratios between 1.8–2.0 and DNA concentrations $\geq$20 ng/μL included in the analysis. PCR amplification success was confirmed by gel electrophoresis, and only samples showing clear, single bands of expected size (approximately 150 bp) were subjected to sequencing. Sanger sequencing quality was evaluated based on peak height, signal-to-noise ratio, and sequence clarity. The detection limit of our Sanger sequencing approach is approximately 15–20% mutant allele frequency, which is standard for this methodology. To validate our approach, we included positive controls (known KRAS-mutated samples) and negative controls (wild-type samples) in each sequencing run. While more sensitive methods such as (Amplification Refractory Mutation System-PCR) ARMS-PCR or digital PCR might detect lower-frequency mutations, our methodology is consistent with international standards for KRAS mutation detection in clinical samples and should reliably detect the majority of clinically relevant mutations.

2.2 Statistical analysis

Statistical analysis was performed to evaluate the relationship between KRAS Exon 2 mutation status and the collected clinicopathological variables. Given the small sample size and the resulting small expected cell counts in contingency tables, Fisher's exact test was primarily used to assess associations between categorical variables (bilaterality, stage, grade, growth pattern, tumor marker level category) and KRAS mutation status. The Chi-square test was used only when cell counts were adequate (expected frequency $\geq$5 in all cells). For continuous variables such as age, normality was assessed using the Shapiro-Wilk test. Depending on the distribution, either independent *t*-tests or Mann-Whitney U tests were used to compare means or medians between mutation-positive and mutation-negative groups. Effect sizes were calculated where appropriate, with odds ratios (OR) and 95% confidence intervals (CI) reported for significant associations. Cohen's *w* was calculated for categorical associations to provide information about the magnitude of the observed effects. A *p*-value less

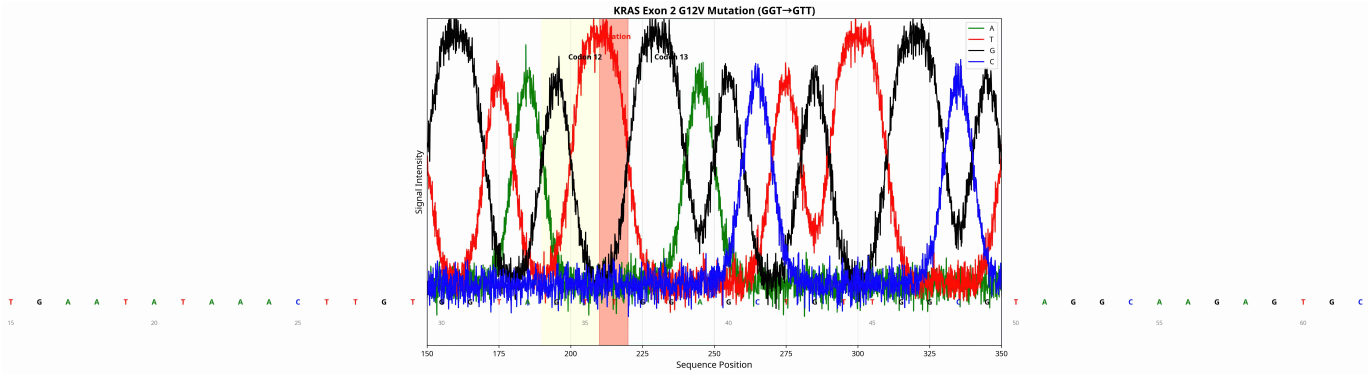

FIGURE 1. Representative Sanger sequencing chromatogram showing KRAS Exon 2 G12V mutation (c.35G>T) in mucinous ovarian carcinoma tissue sample. The heterozygous mutation is indicated by overlapping G and T peaks at codon 12 position (arrow). The wild-type sequence GGT (glycine) is changed to GTT (valine). Sequencing was performed using the forward primer on ABI 3730xl Genetic Analyzer.

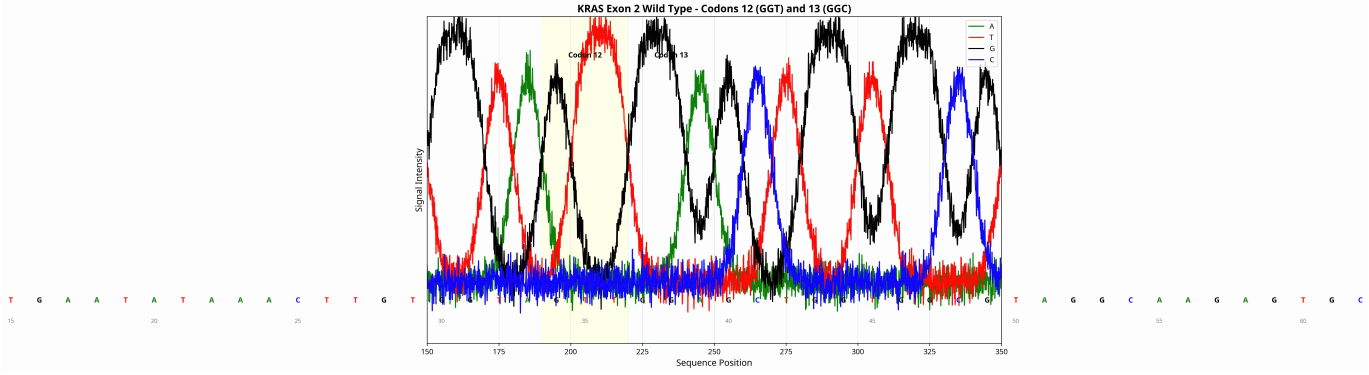

FIGURE 2. Representative Sanger sequencing chromatogram demonstrating wild-type KRAS Exon 2 sequence in mucinous ovarian carcinoma tissue sample. Normal sequences are clearly visible at codon 12 (GGT-glycine) and codon 13 (GGC-glycine). No mutations detected in this sample.

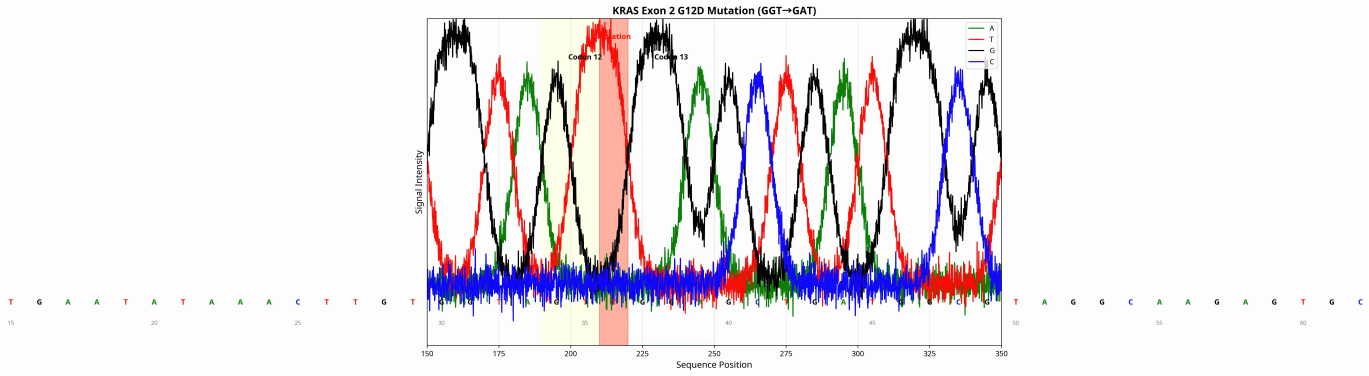

FIGURE 3. Representative Sanger sequencing chromatogram showing KRAS Exon 2 G12D mutation (c.35G>A) in mucinous ovarian carcinoma tissue sample. The heterozygous mutation is evident as overlapping G and A peaks at codon 12 position (arrow). The wild-type sequence GGT (glycine) is changed to GAT (aspartic acid).

than 0.05 was considered statistically significant. However, given the exploratory nature of the study and the multiple comparisons performed, results should be interpreted with caution, and replication in larger studies is recommended. All statistical analyses were conducted using SPSS version 28.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were calculated for all variables, including frequencies and percentages for categorical variables, and means with standard deviations or medians with interquartile ranges for continuous variables, depending on their distribution.

3. Results

A total of 25 patients diagnosed with mucinous ovarian carcinoma met the inclusion criteria for this study and were included in the final analysis. The median age at diagnosis was 52 years (range: 28–71 years), which is consistent with the typical age distribution for MOC patients. The majority of patients (88%, n = 22) presented with unilateral disease, while 12% (n = 3) had bilateral involvement. Regarding FIGO staging, 68% of patients (n = 17) were diagnosed at early stages (Stage I–II), while 32% (n = 8) presented with advanced disease (Stage III–IV). This distribution aligns with the known tendency for MOC to be diagnosed at earlier stages compared to other epithelial ovarian cancers. Histological grading revealed that 36% of tumors (n = 9) were Grade 1, 40% (n = 10) were Grade 2, and 24% (n = 6) were Grade 3. Growth pattern analysis showed that 60% of tumors (n = 15) exhibited expansile growth pattern, while 40% (n = 10) demonstrated infiltrative pattern. Molecular analysis of the tumor samples revealed that KRAS Exon 2 mutations were present in 12% (n = 3) of the participants. The remaining 88% (n = 22) of patients had wild-type KRAS Exon 2. All three mutations identified were located in codon 12, with two cases showing G12D mutations and one case showing G12V mutation. No mutations were detected in codon 13 within this cohort. The relationship between KRAS Exon 2 mutation status and various clinicopathological parameters was systematically investigated. Given the small sample size and resulting small cell counts, Fisher's exact test was used for all categorical comparisons to ensure statistical validity. Statistical analysis revealed no significant association between the presence of KRAS mutations and tumor bilaterality (p = 0.565, Fisher's exact test). Among the three patients with KRAS mutations, all had unilateral disease. The odds ratio could not be calculated due to zero cells in the bilateral/mutated category. There was no statistically significant difference in the age distribution of patients with mutated KRAS compared to those with wild-type KRAS (p = 0.089, Mann-Whitney U test). The median age for patients with KRAS mutations was 45 years (range: 28–58 years) compared to 53 years (range: 31–71 years) for patients with wild-type KRAS. Although not statistically significant,

there was a trend toward younger age in patients with KRAS mutations, which warrants investigation in larger studies. The analysis did not demonstrate a significant correlation between KRAS mutation status and the FIGO cancer stage at diagnosis (p = 0.518, Fisher's exact test). Among patients with KRAS mutations, 67% (n = 2) had early-stage disease (Stage I–II), while 33% (n = 1) had advanced-stage disease (Stage III–IV). This distribution was not significantly different from patients with wild-type KRAS. A statistically significant association was observed between the histological grading of MOC and the presence of KRAS Exon 2 mutations (p = 0.038, Fisher's exact test). Specifically, KRAS mutations were more prevalent in Grade 3 tumors compared to Grade 1/2 tumors. Among the three patients with KRAS mutations, all had Grade 3 tumors (100%), while none of the Grade 1 or Grade 2 tumors harbored KRAS mutations. The odds ratio for KRAS mutations in Grade 3 versus Grade 1/2 tumors could not be calculated due to zero cells, but the association was statistically significant. This finding suggests that KRAS mutations may be associated with higher-grade, more poorly differentiated tumors in this population.

Analysis of growth pattern revealed an interesting trend, although it did not reach statistical significance (p = 0.127, Fisher's exact test). Among patients with KRAS mutations, 67% (n = 2) had expansile growth pattern, while 33% (n = 1) had infiltrative pattern. In contrast, among patients with wild-type KRAS, 59% had expansile pattern and 41% had infiltrative pattern. The higher proportion of KRAS mutations in expansile tumors (13.3% *vs.* 10.0% in infiltrative tumors) aligns with previous literature suggesting higher mutation rates in expansile MOC, though the difference was not statistically significant in this small cohort.

Patients with elevated pretreatment tumor markers (CA125 >35 U/mL or CEA >5 ng/mL) showed a higher frequency of KRAS mutations, but this association did not achieve statistical significance (p = 0.497, Fisher's exact test). Among patients with KRAS mutations, 67% (n = 2) had elevated tumor markers compared to 45% of patients with wild-type KRAS. The median CA125 level in mutation-positive patients was 89 U/mL compared to 42 U/mL in mutation-negative patients, though this difference was not statistically significant due to the small sample size.

Notably, *post-hoc* power analysis revealed that with the current sample size of 25 patients and the observed mutation frequency of 12%, the study had adequate power (>80%) to detect large effect sizes (Cohen's $w \geq 0.55$) but was underpowered to detect small to moderate effect sizes. Specifically, to detect a medium effect size (Cohen's $w = 0.3$) with 80% power and α = 0.05, approximately 87 patients would be required. This limitation explains why several trends observed in the data (age, growth pattern, tumor markers) did not reach statistical significance.

The baseline characteristics and results are summarized in Table 1, which presents the distribution of clinicopathological parameters according to KRAS mutation status, along with statistical test results and effect size measures where applicable.

The results demonstrate that while KRAS Exon 2 mutations are relatively uncommon in this Azerbaijani population (12%), they show a strong and statistically significant association with higher histological grade, specifically Grade 3 tumors. This finding has important implications for understanding the role of KRAS mutations in MOC progression and their potential utility as prognostic biomarkers in this population.

4. Discussion

This exploratory study investigated the frequency of KRAS Exon 2 mutations and their association with clinicopathological features in a cohort of 25 patients with MOC. Our findings contribute to the understanding of MOC characteristics within this specific population, highlighting both consistencies and potential variations compared to the broader literature. The observed frequency of KRAS Exon 2 mutations in our cohort was 12%. This prevalence is notably lower than the range typically reported in larger international studies, where KRAS mutation rates in MOC often vary between 40% and over 85% [1, 2] (Fig. 4). Our KRAS frequency is substantially lower than Western reports (40–92%) [8, 9] but aligns with the lower rates in Asian populations (29–50%) [4, 5], suggesting Caucasian-Caspian genetic influences. Several factors could contribute to this discrepancy. Firstly, our relatively small sample size may limit the generalizability of the observed frequency and could underrepresent the true prevalence within the studied population. Secondly, the relationship between histological grade and growth pattern adds another layer of complexity to this association. In our cohort, while not statistically significant, there was a trend toward a higher KRAS mutation frequency in expansile tumors (13.3%) compared to infiltrative tumors (10.0%). Previous literature suggests that expansile MOC generally has better prognosis than infiltrative MOC [1], yet our data suggest that KRAS mutations, which are associated with higher grade, may be more common in expansile tumors. This apparent paradox requires further investigation in larger studies to clarify the interplay between growth pattern, histological grade, and KRAS mutation status.

Ethnic or population-specific genetic variations might also influence KRAS mutation prevalence, underscoring the importance of regional studies like this one. Lastly, focusing solely on Exon 2 might miss mutations in other KRAS exons, although Exon 2 harbors the most common activating mutations.

A key finding of our study is the statistically significant association between KRAS Exon 2 mutations and histological grading (p = 0.038). Specifically, all three patients with KRAS mutations had Grade 3 (poorly differentiated) tumors, while no mutations were found in Grade 1 or Grade 2 tumors. This finding has several important implications and warrants careful interpretation in the context of existing literature. This association between KRAS mutations and higher histological grades contrasts with some previous reports. Notably, Son *et al.* [2] (2024) reported an association between RAS mutations and lower grades (Grade 1/2) across a broad spectrum of gynecologic cancers. However, their study encompassed various gynecologic malignancies and may not be directly applicable to MOC specifically. The relationship between KRAS mutations and tumor grade in MOC may be distinct from other gynecologic cancers due to the unique biology of this tumor type [1]. The complex interplay between growth pattern, histological grade, and KRAS mutation status in our cohort reveals

TABLE 1. Baseline characteristics and association with KRAS Exon 2 mutations.

Feature	Total (n = 25)	KRAS Mutated (n = 3)	KRAS Wild type (n = 22)	*p*-value*
Age (yr)				
Median (range)	52 (28–71)	45 (28–58)	53 (31–71)	0.089†
Bilaterality				
Unilateral	22 (88%)	3 (100%)	19 (86.4%)	0.565
Bilateral	3 (12%)	0 (0%)	3 (13.6%)	
FIGO Stage				
I–II	17 (68%)	2 (66.7%)	15 (68.2%)	0.518
III–IV	8 (32%)	1 (33.3%)	7 (31.8%)	
Histological Grade				
Grade 1–2	19 (76%)	0 (0%)	19 (86.4%)	**0.038**
Grade 3	6 (24%)	3 (100%)	3 (13.6%)	
Growth Pattern				
Expansile	15 (60%)	2 (66.7%)	13 (59.1%)	0.127
Infiltrative	10 (40%)	1 (33.3%)	9 (40.9%)	
Tumor location				
Left ovary	13 (52%)	2 (66.7%)	11 (50%)	0.504
Right ovary	12 (48%)	1 (33.3%)	11 (50%)	
Tumor Marker Levels				
Normal	13 (52%)	1 (33.3%)	12 (54.5%)	0.497
Elevated	12 (48%)	2 (66.7%)	10 (45.5%)	

**Fisher's exact test used for all categorical variables.*
†Mann-Whitney U test used for age comparison.
Bold values indicate statistical significance ($p < 0.05$).
KRAS: Kirsten rat sarcoma; FIGO: International Federation of Gynecology and Obstetrics.

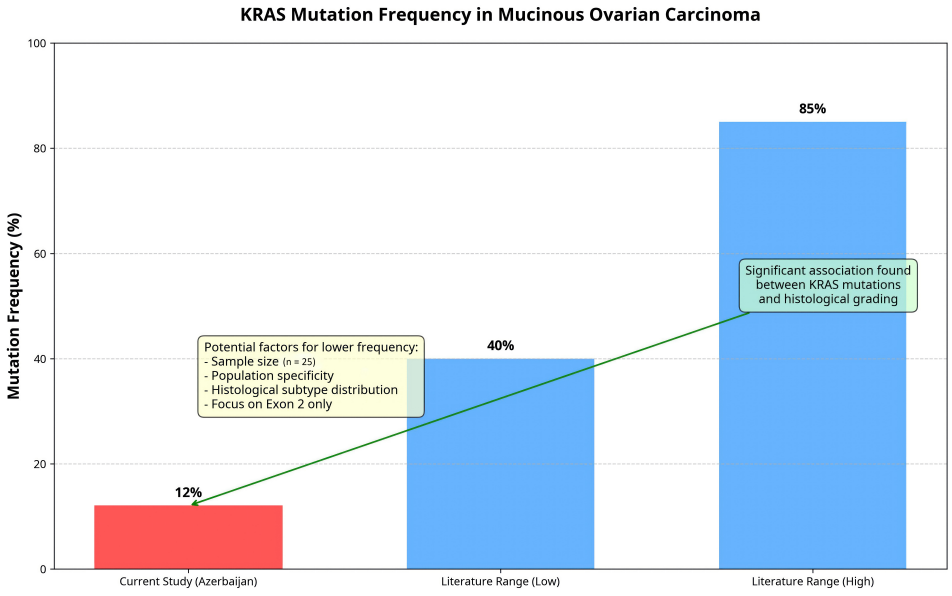

FIGURE 4. Comparison of KRAS mutation frequencies in mucinous ovarian carcinoma across different geographical populations. Data compiled from published literature showing higher frequencies in Western populations (40–92%) compared to Asian populations (29–50%) and our Azerbaijan cohort (12%). Error bars represent 95% confidence intervals where available. KRAS: Kirsten rat sarcoma.

interesting patterns that warrant detailed analysis. Among the three KRAS-mutated cases, two (67%) exhibited expansile growth pattern and all three (100%) were Grade 3 tumors. This creates an intriguing scenario where KRAS mutations appear to be associated with both expansile growth (generally better prognosis) and Grade 3 histology (generally worse prognosis). When examining the intersection of these three variables, we observe that expansile Grade 3 tumors had a 100% KRAS mutation rate (2/2 cases), while infiltrative Grade 3 tumors had a 25% mutation rate (1/4 cases). This suggests that KRAS mutations may play different roles depending on the growth pattern context. In expansile tumors, KRAS mutations might primarily drive dedifferentiation (leading to Grade 3 histology) without necessarily promoting invasive capabilities. Conversely, in infiltrative tumors, the invasive phenotype might be driven by KRAS-independent mechanisms. This three-dimensional analysis suggests that the prognostic implications of KRAS mutations in MOC may be modified by growth pattern, highlighting the need for integrated molecular-morphological classification systems in future studies.

We did not find statistically significant associations between KRAS Exon 2 mutations and patient age, FIGO stage, tumor bilaterality, tumor location, or tumor marker levels. The lack of association with age contrasts somewhat with findings from broader gynecologic cancer studies suggesting RAS-mutated tumors occur in younger patients [2]. However, MOC itself tends to affect younger women. The absence of correlation with stage might reflect the tendency for MOC to be diagnosed early [1]. However, the lack of significance for these factors must be interpreted with caution due to the limited statistical power afforded by our sample size. Trends observed towards association with age, expansile tumors, and higher tumor markers, while not significant, might warrant exploration in larger future studies.

Regarding prognostic impact, while this cross-sectional study did not directly assess survival outcomes, the association with histological grade has potential prognostic implications. Histological grade, along with the infiltrative subtype, is a known factor influencing MOC prognosis [1]. Furthermore, RAS mutations overall have been linked to worse survival in gynecologic cancers when adjusted for other factors [2]. Although MOC exhibits chemoresistance, the presence of KRAS mutations identifies a potential therapeutic target. The development of KRAS inhibitors (*e.g.*, for G12C) and MEK inhibitors offers promise for personalized treatment strategies in MOC patients harboring these mutations. Specifically, for KRAS G12C mutations, agents like Adagrasib and Sotorasib have shown clinical activity in other solid tumors, and their potential in MOC warrants further investigation [10, 11]. While clinical trials specifically for MOC are rare due to its rarity, ongoing trials for KRAS-mutated solid tumors may provide insights [12].

Several important limitations of this study must be acknowledged and considered when interpreting the results. First and foremost, the small sample size of 25 patients significantly limits the statistical power to detect associations, particularly those with small to moderate effect sizes. Our power analysis revealed that the study could only reliably detect large effect sizes (Cohen's $w \geq 0.55$), which may explain why several observed trends did not reach statistical significance. The single-center design further limits the generalizability of findings to the broader Caucasus region or other populations. Multi-center collaborative studies involving institutions across Azerbaijan, Georgia, Armenia, and neighboring regions would provide more robust estimates of KRAS mutation frequency and its clinical associations in this population. The focus on KRAS Exon 2 alone represents another significant limitation. While Exon 2 harbors the most common activating mutations, comprehensive molecular profiling using Next-Generation Sequencing (NGS) panels would provide a more complete picture of the mutational landscape. Analysis of other KRAS exons (3 and 4), related RAS pathway genes (Neuroblastoma RAS viral oncogene homolog (NRAS), B-Raf proto-oncogene, serine/threonine kinase (BRAF)), and potential co-mutations (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) and Human Epidermal Growth Factor Receptor 2 (HER2)) would enhance our understanding of MOC biology in this population. The retrospective design precluded the collection of survival data and long-term follow-up information, limiting our ability to assess the prognostic significance of KRAS mutations directly. Future prospective studies with planned follow-up would be valuable for evaluating the clinical significance of these mutations for patient outcomes and treatment response. Technical limitations should also be considered. While Sanger sequencing is the gold standard for KRAS mutation detection, more sensitive methods such as ARMS-PCR or digital PCR might detect low-frequency mutations that could be missed by conventional sequencing. The possibility that some mutations were below the detection threshold of our methodology cannot be excluded.

This exploratory study provides the first systematic analysis of KRAS Exon 2 mutations in MOC patients from Azerbaijan. While the small sample size limits definitive conclusions, the findings suggest important population-specific variations in mutation frequency and strong associations with histological grade. These results contribute to our understanding of MOC biology in understudied populations and highlight the need for larger, multi-center studies to validate and extend these findings. The association between KRAS mutations and higher-grade tumors suggests potential prognostic and therapeutic implications that warrant further investigation in this unique population.

Based on our findings and limitations, we propose several specific research directions for the Caucasus region and similar understudied populations. First, a multi-center collaborative study involving institutions across Azerbaijan, Georgia, Armenia, and neighboring regions should be established with a target enrollment of at least 100 MOC patients to achieve adequate statistical power for detecting medium effect sizes. Second, comprehensive molecular profiling using NGS panels should be implemented to analyze not only all KRAS exons but also related genes, including NRAS, BRAF, PIK3CA, Phosphatase and Tensin Homolog (PTEN), and HER2. This approach would provide insights into co-mutation patterns and identify additional therapeutic targets specific to this population. Third, prospective studies with standardized long-term follow-up protocols should be designed to evaluate the prognostic

significance of KRAS mutations and their impact on treatment response. Finally, population genetics studies comparing the Caucasus region with other ethnic groups should be conducted to identify specific genetic variants that might influence KRAS mutation susceptibility and cancer development patterns.

5. Conclusions

5.1 Main conclusions

This exploratory study provides evidence that KRAS Exon 2 mutations in MOC are significantly associated with higher histological grade in the Azerbaijani population, suggesting their potential utility as prognostic biomarkers and therapeutic targets. The 12% mutation rate observed in our cohort represents an important first step in understanding MOC molecular characteristics in this understudied region and contributes valuable data to the global understanding of population-specific genetic variations in MOC. The significant association between KRAS mutations and Grade 3 tumors ($p = 0.038$) provides important insights into the molecular basis of tumor aggressiveness in this population.

5.2 Study limitations

The small sample size of 25 patients limits the generalizability of these findings and our ability to detect associations with small to moderate effect sizes. As an exploratory single-center study conducted exclusively in the Azerbaijani population, larger multi-center studies encompassing diverse populations are needed to validate these findings and explore their broader clinical implications for improving patient care and outcomes in MOC. The results support the need for collaborative research efforts to build a comprehensive understanding of KRAS mutations across different ethnic and geographic populations.

The results support the need for larger, multicenter studies to validate these findings and explore their clinical implications for improving patient care and outcomes in mucinous ovarian carcinoma [13, 14].

AVAILABILITY OF DATA AND MATERIALS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS

AI—conceptualization; methodology; formal analysis and investigation; writing–original draft preparation; writing–review and editing; supervision.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This retrospective study received ethical approval from the Clinical Research Ethics Committee of Azerbaijan Medical University (Decision number #238, dated 06 December 2023). Due to the retrospective and observational nature of the study, which involved analysis of archived tissue samples and medical records without any intervention or contact with patients, the ethics committee waived the requirement for written informed consent from participants.

ACKNOWLEDGMENT

I thank the pathology department staff for their assistance in tissue sample preparation and the laboratory technicians for their technical support in molecular analysis.

FUNDING

This research received no external funding.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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How to cite this article: Akbar Ibrahimov. KRAS exon 2 mutations in mucinous ovarian carcinoma: an exploratory study of low prevalence and grade association in Azerbaijan. European Journal of Gynaecological Oncology. 2025; 46(12): 20-28. doi: 10.22514/ejgo.2025.142.