ORIGINAL RESEARCH ARTICLE

DNA methylation of *ANKRD23* as a novel biomarker for early diagnosis of ovarian cancer

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Abstract

Ovarian cancer (OC) is a leading cause of gynecologic cancer-related mortality and is frequently diagnosed at advanced stages due to the absence of effective early detection tools. Epigenetic alterations, particularly DNA methylation, play a critical role in tumorigenesis and represent promising non-invasive biomarkers. This study investigated the methylation status of the *ANKRD23* gene in OC and evaluated its diagnostic potential using peripheral blood samples. A total of 385 serous OC patients, 50 individuals with benign ovarian conditions, and 99 healthy controls were included. DNA methylation was assessed using methylation-sensitive restriction enzymes followed by real-time PCR. *ANKRD23* methylation was significantly associated with clinical stage ($p = 0.029$), histological grade ($p = 0.009$), and ethnicity ($p = 0.024$). Moreover, methylation levels differed significantly among OC patients, benign cases, and healthy controls ($p = 0.026$). These findings support blood-based *ANKRD23* methylation as a promising biomarker for non-invasive ovarian cancer diagnosis and risk stratification. *(Afr J Reprod Health 2026; 30 [15] 16-25).*

Keywords: Ovarian cancer, DNA methylation, Epigenetic biomarker, *ANKRD23* methylation

Résumé

Le cancer de l'ovaire (CO) est l'une des principales causes de mortalité liée aux cancers gynécologiques et est souvent diagnostiqué à des stades avancés en raison de l'absence d'outils efficaces de détection précoce. Les altérations épigénétiques, en particulier la méthylation de l'ADN, jouent un rôle clé dans la tumorigénèse et représentent des biomarqueurs non invasifs prometteurs. Cette étude a évalué le statut de méthylation du gène ANKRD23 dans le cancer de l'ovaire et son potentiel diagnostique à partir d'échantillons de sang périphérique. Au total, 385 patientes atteintes d'un cancer séreux de l'ovaire, 50 individus présentant des pathologies ovariennes bénignes et 99 témoins sains ont été inclus. La méthylation de l'ADN a été analysée à l'aide d'enzymes de restriction sensibles à la méthylation, suivie d'une PCR en temps réel. La méthylation d'ANKRD23 était significativement associée au stade clinique ($p = 0,029$), au grade histologique ($p = 0,009$) et à l'origine ethnique ($p = 0,024$). De plus, les niveaux de méthylation différaient significativement entre les patientes atteintes de cancer de l'ovaire, les cas bénins et les témoins sains ($p = 0,026$). Ces résultats suggèrent que la méthylation d'ANKRD23 détectée dans le sang constitue un biomarqueur prometteur pour le diagnostic non invasif et la stratification du risque du cancer de l'ovaire. *(Afr J Reprod Health 2026; 30 [15]:16-25).*

Mots-clés: Cancer de l'ovaire, Méthylation de l'ADN, Biomarqueur épigénétique, Méthylation d'ANKRD23

Introduction

Ovarian cancer (OC) is a significant global health concern, ranking as the eighth most common gynecological malignancy and the fifth leading cause of cancer-related mortality among women worldwide.[1] The absence of specific early symptoms and the lack of reliable diagnostic tools often result in late-stage diagnoses, where treatment options are limited, and outcomes are poor.[2,3] While early-stage OC has a favorable 5-year survival rate of approximately 90%, this figure drops dramatically to 20-40% in advanced-stage cases.[4] Given the aggressive nature of OC and its poor

prognosis when diagnosed late, identifying reliable biomarkers for early detection and targeted therapies is a critical priority. DNA methylation, a key epigenetic modification, is pivotal in regulating gene expression and maintaining cellular homeostasis.[5-7] Aberrant methylation patterns, such as promoter hypermethylation that silences tumor suppressor genes (TSGs) and global hypomethylation that contributes to genomic instability, are hallmarks of various cancers, including OC.[8,9] These epigenetic alterations impact key pathways involved in tumor initiation, progression, and metastasis.[10] In OC, specific methylation profiles have been linked to the inactivation of TSGs and dysregulation of oncogenes, highlighting their potential as diagnostic and prognostic biomarkers.[11,12] Investigating these methylation patterns provides valuable insights into OC pathogenesis and offers opportunities for the development of novel early detection methods and targeted therapeutic approaches.

Ankyrins are scaffold proteins that facilitate communication between membrane-associated proteins and the cytoskeleton, thereby regulating cellular structure and signaling. Beyond their established roles in muscle and nervous system development, increasing evidence suggests that aberrant ankyrin expression or function contributes to the pathogenesis of multiple diseases, including cancer, where they may influence cell proliferation, migration, and survival.[13] The *ANKRD23* gene, located at 1p36.33 on chromosome 1, encodes a protein belonging to the ankyrin repeat domain-containing family, which is involved in critical cellular processes including signal transduction, protein-protein interactions, and stress responses.[14] Members of the ankyrin repeat domain-containing (ANKRD) protein family have been increasingly implicated in cancer biology, where they appear to exert context-dependent functions across different tumor types. Computational analyses performed by Colaprico et al. identified *ANKRD23* as a potential cancer-related gene and suggested that its biological role may vary according to tissue context, acting as a putative oncogene in clear-cell renal cell carcinoma while exhibiting tumor-suppressive characteristics in other malignancies.[15] These findings suggest that ANKRD proteins may contribute to tumor initiation and progression through multiple molecular mechanisms. However, despite growing interest in the ANKRD family, the biological significance of *ANKRD23* in OC remains largely unexplored, and its epigenetic regulation has not been adequately investigated.

Additionally, the ability of *ANKRD* genes to regulate the cell cycle via transcription factors like E2F1 underscores their significant influence on cancer biology.[16] A deeper understanding of the mechanisms by which *ANKRD* genes contribute to cancer progression across different contexts could provide valuable insights for developing targeted therapies.

High-grade serous ovarian cancer (HGSC), the most lethal subtype of OC[17], further highlights the significance of identifying genes that may hold prognostic and therapeutic value.[18] The present study aimed to investigate the methylation levels of the *ANKRD23* gene in patients with serous ovarian cancer (SOC), individuals with benign ovarian conditions**,** and healthy controls. Building on our previous research, which involved comprehensive genomic profiling of a family cohort, including a SOC patient and her healthy monozygotic twin sibling, we identified differential methylation patterns in blood and tissue samples that distinguished individuals affected by cancer from those who were unaffected.[19,20]

Among the genes analyzed, *ANKRD23* emerged as a candidate with a distinct methylation profile in the blood samples of affected individuals, suggesting its potential role in OC pathogenesis. In this study, we observed elevated levels of *ANKRD23* methylation in the plasma samples of patients with SOC compared to individuals with benign ovarian conditions and healthy controls. While our initial research focused on the concordance of methylation patterns in blood samples, the current study builds on these findings by assessing *ANKRD23* methylation in a larger cohort using peripheral blood samples. These results suggest a significant relationship between *ANKRD23* methylation and the biological mechanisms underlying the development and progression of OC. Additionally, our findings

underscore the potential of *ANKRD23* methylation as a diagnostic biomarker to differentiate between ovarian cancer and benign ovarian conditions.

Methods

Study population

This study involved 385 patients with SOC, 50 individuals with benign ovarian conditions, and 99 healthy controls matched for age, gender, and ethnicity. Blood samples were collected from patients who visited the Cancer Genetics Department at the Istanbul University Oncology Institute between 2021 and 2023. All participants provided written informed consent before sample collection. Cancer genetics specialists conducted genetic counseling, and clinical data were collected during the initial genetic counseling session, which occurred at the time of genetic testing. The study was conducted at the Cancer Genetics Lab, Istanbul University Oncology Institute, Türkiye.

DNA extraction and methylation analysis

DNA was extracted from peripheral blood lymphocytes, and samples from SOC patients were collected before surgery and before the initiation of chemotherapy. For methylation analysis, blood samples were preserved in liquid nitrogen (-196°C) and ultra-low-temperature freezers (-80°C) until further processing.

Methylation-sensitive restriction enzymes (MSREs)

DNA extraction from blood samples, including those from the patient cohort, healthy controls, and individuals with benign ovarian conditions, was performed using the *Quick-DNA™ Miniprep Plus Kit* (Zymo Research, CA, USA) following the manufacturer's instructions. The concentration of the extracted DNA was measured using a Nanodrop 2000 spectrophotometer (Thermo Fisher, USA), and the integrity of the DNA was evaluated via electrophoresis on a 0.8% agarose gel. DNA methylation analysis was conducted with the *OneStep qMethyl™ Kit* (Zymo Research, CA, USA). Real-time PCR amplification was performed using SYTO® 9 fluorescent dye and gene-specific primers for *ANKRD23* sense: *5'-GCA GAA GCT TGA CTG CCT-3'; antisense: 5'-AAT GGT GGC AGT GCG AAA-3',* designed with the Integrated DNA Technologies Oligoanalyzer tool. The *Zymo Research OneStep qMethyl Kit* was used with the Applied Biosystems Real-Time PCR system (Thermo Fisher, USA). Each sample was analyzed in triplicate. The PCR protocol involved an initial digestion step with MSREs at 37°C for 2 hours, followed by denaturation at 95°C for 10 minutes. Amplification consisted of 40 cycles of denaturation at 95°C for 10 seconds, annealing at 54°C for 60 seconds, and extension at 72°C for 60 seconds, followed by a final elongation step at 72°C for 7 minutes. Each reaction contained 10 µL of 2X preMix, 5 µL of DNA sample, 2 µL of each primer (sense and antisense), and DNase/RNase-free water for a total reaction volume of 20 µL. Cycle threshold (Ct) values for the test and reference reactions were recorded during amplification. Methylation percentages for the *ANKRD23* gene were calculated using the Δ^{Ct} method with the formula 100 x $2^{-\Delta\Delta Ct}$, where ΔCt represents the difference between the target Ct and the reference Ct. Samples with methylation levels of ≤ 6 % were classified as unmethylated, whereas those exceeding 6% were categorized as methylated. As for the 6% methylation threshold, this value was predefined by the manufacturer of the Zymo Research OneStep qMethyl™ Kit Manual (Zymo Research Corp, Irvine, CA, USA) [21] used in this study. The kit's guidelines specify that 6% should be used as the reference threshold for methylation analysis, and all assessments were conducted by this standardized recommendation.

Statistical analysis

Descriptive statistics for qualitative variables were presented as numbers and percentages, while quantitative variables were summarized using the mean, standard deviation, median, minimum, and maximum values. Associations between qualitative variables were assessed using Pearson's Chi-square test. The normality of the distribution of quantitative variables was evaluated using the Shapiro-Wilk test. The Kruskal-Wallis test was used to compare the distrubutions of quantative variables among three independent groups. Post hoc investigations were made with the Dunn test. A p-value of less than 0.05 was considered statistically

significant. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), version 28(IBM Corp., Armonk, NY).

Ethical approval and informed consent statements

The Istanbul Faculty of Medicine Ethics Committee approved the study (Ethics Committee Approval: 2021/789-2019/1161). Blood samples were drawn after obtaining informed consent from patients and healthy controls. The study was conducted in accordance with the principles of the Declaration of Helsinki.

Results

Clinicopathologic characteristics of the study cohort

The clinicopathologic characteristics of the study cohort are summarized in Figure 1. Most OC patients were older than 45 years (67.5%), whereas only 19.5% were aged 45 years or younger. Advanced-stage disease predominated within the cohort, with 52.8% and 13.2% of patients diagnosed at Stage III and Stage IV, respectively, while only 15.8% were diagnosed at Stage I. Histopathological evaluation revealed that the majority of tumors were of high grade, with Grade 3 tumors accounting for 50.4% of cases, followed by Grade 2 tumors (26.5%). The study population was predominantly of Turkish origin (68.3%), with smaller proportions originating from the Balkan Region (18.7%) and Eastern Anatolia (9.1%). All OC cases included in the study were classified as serous carcinoma. Analysis of menopausal status demonstrated a comparable distribution between premenopausal (41.8%) and postmenopausal (43.4%) patients.

Table 1 summarizes the associations between *ANKRD23* methylation status and clinicopathological characteristics in OC patients. *ANKRD23* methylation was significantly associated with histological grade ($p = 0.009$), with methylation frequencies increasing from 35.1% in Grade 1 tumors to 48.0% and 56.2% in Grade 2 and Grade 3 tumors, respectively. A significant association was also observed with clinical stage ($p = 0.029$), as methylation was more frequent in advanced-stage tumors, increasing from 41.0% in Stage I to 56.9% in Stage IV disease. Furthermore, methylation status differed significantly according to ethnicity ($p = 0.024$), with Turkish patients representing the largest proportion of methylated cases. In contrast, no significant associations were identified between *ANKRD23* methylation status and age or menopausal status ($p > 0.05$). Data are presented as n (%). Percentages were calculated within each clinicopathological category. Associations were evaluated using Pearson's chi-square test. Cases with missing or unknown clinicopathological information were excluded from the table to improve clarity and readability.

ANKRD23 methylation across study groups

Table 2 presents the comparison of *ANKRD23* methylation levels among healthy controls, individuals with benign ovarian conditions, and OC patients. Significant differences in methylation levels were observed among the study groups ($p = 0.026$). OC patients exhibited the highest methylation levels, with a mean value of 8.85 and a median of 5.75, followed by healthy controls (mean: 7.65, median: 4.09). Individuals with benign ovarian conditions showed the lowest methylation levels (mean: 4.65, median: 3.87). In addition, OC patients demonstrated the widest range of methylation values (0.09–99.20), whereas the benign ovarian condition group showed a narrower distribution (0.67–11.81). These findings indicate marked differences in *ANKRD23* methylation profiles among the study groups, with OC patients exhibiting higher and more heterogeneous methylation levels than the comparison groups. The distribution of *ANKRD23* methylation status across the study groups is presented in Figure 2. Among ovarian cancer patients (n = 385), 193 (50.1%) exhibited *ANKRD23* methylation, whereas 192 (49.9%) were classified as unmethylated. In contrast, methylation was detected in only 13 of 50 individuals with benign ovarian conditions (26.0%), while 37 (74.0%) remained unmethylated. Similarly, among healthy controls (n = 99), 29 (29.3%) showed methylation and 70 (70.7%) were unmethylated. The frequency of *ANKRD23* methylation was therefore markedly higher in ovarian cancer patients than in individuals with

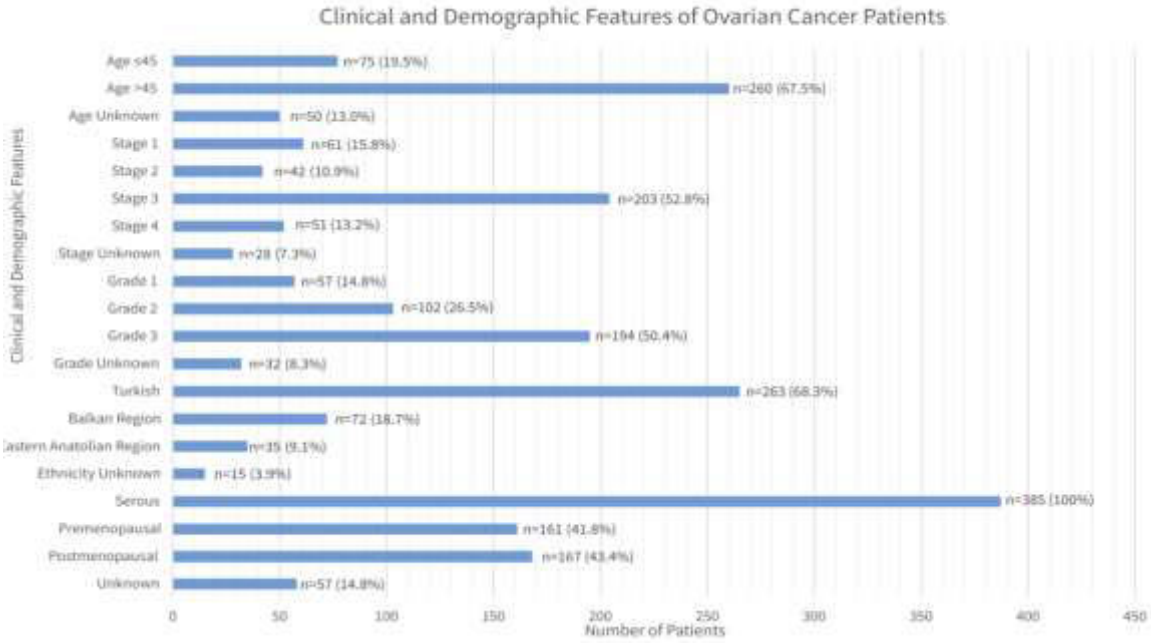

Figure 1: Clinical and demographic features of ovarian cancer patients. (n=Number)

Table 1: Association between *ANKRD23* methylation status and clinicopathological characteristics in OC patients.

Variable	Category	Methylated n (%)	Unmethylated n (%)	p-value
Age	**≤45 years**	33 (44.0)	42 (56.0)	0.400
	>45 years	140 (53.8)	120 (46.2)	
Clinical stage	**Stage I**	25 (41.0)	36 (59.0)	0.029
	Stage II	18 (42.9)	24 (57.1)	
	Stage III	109 (53.7)	94 (46.3)	
	Stage IV	29 (56.9)	22 (43.1)	
Histological grade	**Grade 1**	20 (35.1)	37 (64.9)	0.009
	Grade 2	49 (48.0)	53 (52.0)	
	Grade 3	109 (56.2)	85 (43.8)	
Ethnicity	**Turkish**	134 (51.0)	129 (49.0)	0.024
	Balkan Region	38 (52.8)	34 (47.2)	
	Eastern Anatolian Region	18 (51.4)	17 (48.6)	
Menopausal status	**Premenopausal**	80 (49.7)	81 (50.3)	0.760
	Postmenopausal	84 (50.3)	83 (49.7)	

Table 2: Comparison of *ANKRD23* gene methylation levels among study groups.

Study Group	n	Mean	Median	SD	Minimum	Maximum	p-value
Healthy Controls	99	7.65	4.09	13.35	0.07	98.40	
Benign Ovarian Conditions	50	4.65	3.87	2.75	0.67	11.81	0.026
Ovarian Cancer Patients	385	8.85	5.75	10.99	0.09	99.20	

Data are presented as mean, median, standard deviation (SD), minimum, and maximum methylation levels. Differences among study groups were evaluated using the Kruskal–Wallis test. OC patients exhibited the highest *ANKRD23* methylation levels compared with individuals with benign ovarian conditions and healthy controls (p = 0.026). SD: standard deviation

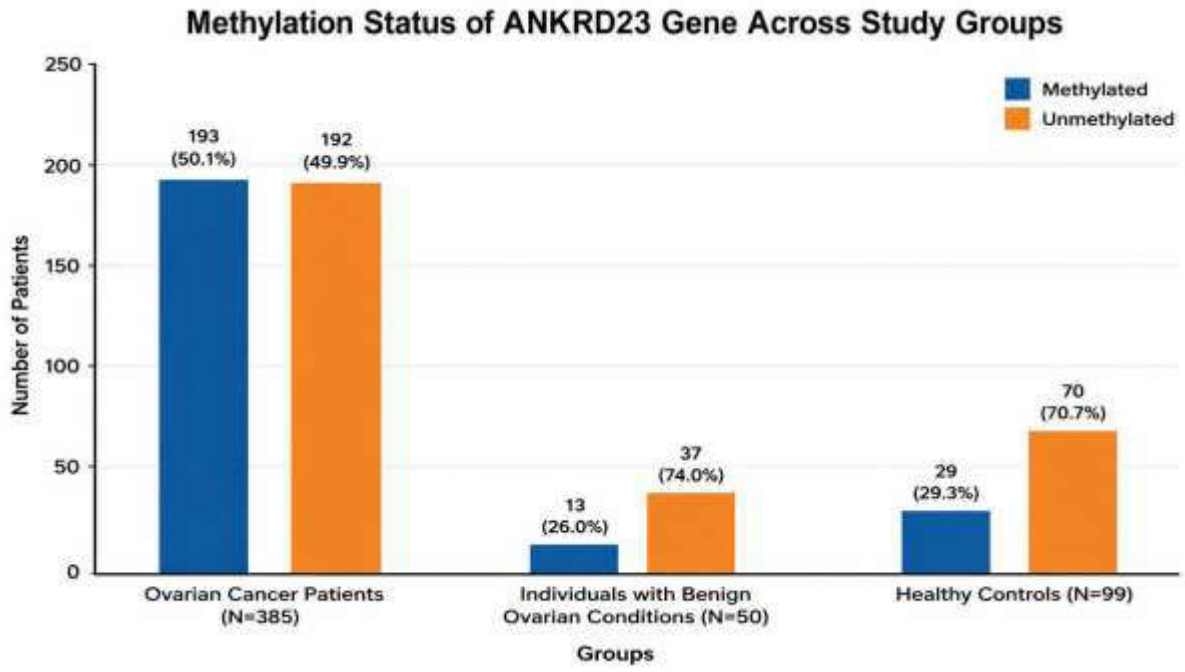

Figure 2: Distribution of *ANKRD23* methylation status among study groups .(N: Number)

benign ovarian conditions and healthy controls, demonstrating distinct methylation patterns among the study groups

Discussion

Genetic and epigenetic alterations are integral to the development and progression of various cancers, including OC.[22,23] Among these, aberrant promoter hypermethylation is a well-documented epigenetic modification that silences tumor suppressor genes (TSGs) and represents an early event in carcinogenesis.[24,25] The detection of such epigenetic changes in cell-free circulating DNA from blood and other body fluids has garnered increasing attention due to its non-invasive nature and accessibility, offering significant potential for early diagnosis and cancer monitoring.[26-28]

Recent studies have increasingly focused on epigenetic changes in cancer, with DNA methylation being the first discovered in cancer cells.[29, 30] DNA methylation involves the addition of a methyl group to the carbon-5 position of a cytosine molecule, a process facilitated by DNA methyltransferase enzymes.[31] This change happens in specific DNA sequences called CpG dinucleotides (5'-CG-3') and plays a key role in controlling gene expression.

Disruptions in this process are common in cancer and contribute to its development.[32] In this study, we investigated the methylation profile of the *ANKRD23* gene in SOC patients, comparing it to individuals with benign ovarian conditions and healthy controls. Our findings revealed an increase in *ANKRD23* methylation levels in OC patients ($p = 0.026$), highlighting its potential utility as a biomarker for differentiating malignant from benign conditions. Unlike prior research that predominantly analyzed tumor tissue samples, we employed blood-based samples, offering a non-invasive method to assess circulating tumor DNA (ctDNA). This approach is consistent with current literature, which underscores the reliability of blood-derived methylation analyses as a surrogate for detecting tumor-specific epigenetic alterations.[33] Our results contribute to the growing body of evidence supporting the use of liquid biopsies for the identification of epigenetic biomarkers in OC.

We observed a correlation between *ANKRD23* methylation and advanced-stage OC. Patients with Stages III and IV disease exhibited

higher methylation levels compared to those in earlier stages. This suggests that *ANKRD23* hypermethylation may be associated with tumor progression, consistent with previous reports linking epigenetic alterations to aggressive tumor phenotypes.[34] The increasing methylation levels from Stage 2 to Stage 4 further support its potential role in disease advancement.

An association was also noted between histological grade and *ANKRD23* methylation in line with previous reports[22]. We found that patients with Grade 3 tumors demonstrated the highest methylation levels, whereas those with lower-grade tumors exhibited a more balanced methylation status. Since higher-grade tumors are generally more aggressive and associated with poorer prognoses[35], our findings suggest that *ANKRD23* hypermethylation may contribute to tumor aggressiveness.

Notably, ethnic background appeared to influence the methylation profiles of genes[36, 37], as observed in *ANKRD23* methylation patterns. In this study, patients from the Turkish, Balkan Region, and Eastern Anatolian Region exhibited differences in methylation status, with higher methylation observed in these populations. These findings suggest that genetic or environmental factors related to ethnicity may contribute to variability in *ANKRD23* methylation. Further studies are warranted to explore whether distinct genetic backgrounds, lifestyle factors, or environmental exposures influence these epigenetic differences.

In this study, we employed a methylation-sensitive restriction enzyme (MSRE)-based qPCR approach to assess the methylation status of the ANKRD23 **gene**. Compared to earlier methods used for methylation analysis, MSRE-based qPCR offers several advantages. [21] It enables methylation-dependent restriction of DNA followed by quantitative PCR assessment of the target region, facilitating the analysis of DNA methylation in limited samples. Additionally, MSRE-based methods allow targeted evaluation of methylation at restriction sites, providing a quantitative assessment of methylation patterns.[38] In this study, we revealed significantly higher methylation levels of the *ANKRD23* gene in OC patients compared to individuals with benign ovarian conditions and healthy controls. The progressive increase in methylation prevalence from healthy controls to OC patients underscores the potential of *ANKRD23* methylation as a biomarker for disease progression. We used peripheral blood samples from 385 OC patients, 50 individuals with benign ovarian conditions, and 99 healthy controls to investigate the *ANKRD23* gene methylation profile. Peripheral blood-based methylation analysis provides a minimally invasive approach for early detection, which is crucial in ovarian cancer due to the absence of an effective screening method.[39,40] However, the functional implications of *ANKRD23* methylation in tumorigenesis remain unclear. Further studies are needed to elucidate its role in regulating gene expression and its interactions with other epigenetic and genetic alterations.

Our study highlights the potential of *ANKRD23* methylation as a biomarker for OC diagnosis. Elevated methylation levels in advanced-stage cases suggest an association with tumor progression. Ethnic differences in methylation patterns emphasize the need for further investigation. Additionally, we acknowledge that gene expression data would strengthen mechanistic insights, and future studies should integrate transcriptomic analysis to enhance understanding. The relationship between *ANKRD23* methylation status and gene expression has not yet been investigated in this study. However, this aspect is planned to be addressed in future research as an independent study. Further analyses will be conducted to investigate whether *ANKRD23* methylation affects its transcriptional activity and the downstream pathways in OC.

Currently, there is limited evidence regarding the role of *ANKRD23* methylation in OC, and to the best of our knowledge, this is among the first studies to investigate its methylation status in peripheral blood samples from patients with SOC. *ANKRD23* was selected based on our preliminary screening analyses, in which it demonstrated a consistent differential methylation pattern between OC cases and controls. The significantly higher methylation levels observed in patients with SOC suggest that epigenetic alterations involving *ANKRD23* may be associated with ovarian tumorigenesis and disease-related biological processes. Although the exact molecular mechanisms remain unclear, these findings support

the potential utility of *ANKRD23* methylation as a complementary biomarker for OC detection and risk stratification. Nevertheless, larger multicenter studies and functional investigations are required to validate these observations and clarify the biological significance of *ANKRD23* methylation in OC. The exclusive focus on *ANKRD23* methylation overlooks other epigenetic mechanisms, such as histone modifications and non-coding RNAs, which may also play a role in OC. Furthermore, advanced methylation analysis techniques, such as bisulfite sequencing, could provide greater accuracy and depth in identifying epigenetic changes. Finally, the lack of long-term follow-up data limits the understanding of how *ANKRD23* methylation impacts disease progression and clinical outcomes.

The investigation of *ANKRD23* methylation as a blood-based biomarker addresses a critical unmet need in OC management. Due to nonspecific symptoms and a lack of reliable early-detection tools, most OC cases are diagnosed at advanced stages, contributing to poor survival rates. This study demonstrates that *ANKRD23* methylation levels in peripheral blood significantly differentiate OC patients from healthy individuals and those with benign conditions. Its association with advanced-stage and high-grade tumors further suggests utility in risk stratification. Validating *ANKRD23* methylation could enable non-invasive, accessible screening for early diagnosis and monitoring, potentially improving survival outcomes in SOC. *ANKRD23* methylation should currently be considered a complementary biomarker rather than a replacement for established ovarian cancer detection methods such as CA-125 and imaging modalities. Its potential clinical value may lie in improving risk stratification and supporting existing diagnostic approaches through a minimally invasive blood-based assessment. Future studies should evaluate its performance in combination with established biomarkers and clinical parameters.

Strengths and limitations

This study has several strengths. To our knowledge, this is the first large-scale study investigating *ANKRD23* methylation in peripheral blood samples from patients with SOC, individuals with benign ovarian conditions, and healthy controls. The inclusion of a relatively large OC cohort, together with benign and healthy comparison groups, enabled a comprehensive evaluation of the diagnostic potential of *ANKRD23* methylation. In addition, the use of a minimally invasive blood-based approach enhances the potential clinical applicability of the findings. Nevertheless, several limitations should be acknowledged.

The study was conducted at a single center, and external validation in independent and ethnically diverse cohorts is required. Furthermore, gene expression analyses and functional experiments were not performed; therefore, the biological mechanisms linking *ANKRD23* methylation to ovarian carcinogenesis remain unclear. Longitudinal follow-up data were also unavailable, preventing assessment of the prognostic value of *ANKRD23* methylation.

From a clinical and policy perspective, our findings support the growing role of blood-based epigenetic biomarkers in OC detection and risk stratification. If validated in future multicenter prospective studies, *ANKRD23* methylation may contribute to the development of non-invasive screening and monitoring strategies, potentially facilitating earlier diagnosis and improving patient management.

Conclusion

In conclusion, *ANKRD23* methylation was significantly associated with ovarian cancer and distinguished patients with ovarian cancer from healthy controls and individuals with benign ovarian conditions. These findings support the potential utility of *ANKRD23* methylation as a minimally invasive blood-based biomarker for ovarian cancer detection and risk stratification. Further multicenter validation and functional studies are required before clinical implementation.

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Author contributions

Aydan Alizada and Ozge Sukruoglu Erdogan contributed to the acquisition, design, and

interpretation of the research study. Seda Kilic Erciyas and Betul Celik Demirbas provided support and advice on technical aspects of the research and drafted the article. Ozge Pasin analyzed the data. Ahmet Dinc helped with the experiments. Pinar Saip and Hulya Yazici selected the patients to be included in this study. Seref Bugra Tuncer supervised the study and critically revised it for important intellectual content.

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Conflict of interest

The authors declare no conflict of interest.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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