Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Genetic Variation in the Oestrogen Pathway and Cancer-Associated Cachexia: Implications for Disease Predictive and Supportive Care

Bernardo Graça Santos
University of Porto (FCUP)
Ana Carolina Leão Silva
Research Centre of IPO Porto (CI-IPOP) / CI- IPOP@RISE (Health Research Network), Portuguese Oncology Institute of Porto (IPO Porto) / Pathology, Porto Comprehensive Cancer Centre Raquel Seruca (Porto.CCC)
Valéria Zuyonak
University of Porto (FCUP)
Cláudia Silva
Portuguese Oncology Institute of Porto (IPO Porto) / Porto Comprehensive Cancer Centre Raquel Seruca (Porto.CCC)
Virgínia Rocha Dias
Portuguese Oncology Institute of Porto (IPO Porto) / Porto Comprehensive Cancer Centre Raquel Seruca (Porto.CCC)
Maria Paula Silva
Portuguese Oncology Institute of Porto (IPO Porto) / Porto Comprehensive Cancer Centre Raquel Seruca (Porto.CCC)
Tiago Ferreira
University of Trás-os-Montes and Alto Douro (UTAD)
Inês Guerra de Melo
Research Centre of IPO Porto (CI-IPOP) / CI- IPOP@RISE (Health Research Network), Portuguese Oncology Institute of Porto (IPO Porto) / Pathology, Porto Comprehensive Cancer Centre Raquel Seruca (Porto.CCC)
Joana M.O. Santos
Research Centre of IPO Porto (CI-IPOP) / CI- IPOP@RISE (Health Research Network), Portuguese Oncology Institute of Porto (IPO Porto) / Pathology, Porto Comprehensive Cancer Centre Raquel Seruca (Porto.CCC)
Rui Medeiros
Research Centre of IPO Porto (CI-IPOP) / CI- IPOP@RISE (Health Research Network), Portuguese Oncology Institute of Porto (IPO Porto) / Pathology, Porto Comprehensive Cancer Centre Raquel Seruca (Porto.CCC)

Valéria Tavares

valeria.tavares@ipoporto.min-saude.pt

Research Centre of IPO Porto (CI-IPOP) / CI- IPOP@RISE (Health Research Network), Portuguese Oncology Institute of Porto (IPO Porto) / Pathology, Porto Comprehensive Cancer Centre Raquel Seruca (Porto.CCC)

Research Article

Keywords: cachexia, hormone, polymorphism, single-nucleotide, biomarkers, supportive care

Posted Date: May 28th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9305705/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Additional Declarations: No competing interests reported.

Abstract

Purpose

Cancer-associated cachexia (CAC) affects over 50% of cancer patients and accounts for approximately 20% of cancer-related deaths, highlighting the need for reliable tools to predict its onset and aid in supportive care. Accumulating evidence suggests that CAC pathogenesis has a strong hormonal component and is also influenced by inherited genetic factors. This study investigated the impact of single-nucleotide polymorphisms (SNPs) in oestrogen-related genes on the development of CAC and overall survival (OS) among cancer patients.

Methods

Three SNPs, namely *ESR1* rs2046210 (G > A), *ESR1* rs9340799 (A > G) and *ESR2* rs928554 (T > C), were genotyped using a TaqMan probe-based approach.

Results

No association was observed between these variants and CAC status in the overall cohort (χ^2 test and Fisher's exact test, $p > 0.05$). However, within the male subgroup, *ESR1* rs2046210 AA genotype carriers exhibited a 49% reduced disease risk [Fearon criteria (with CAC vs. without CAC)] compared with patients with the G allele (Fisher's exact test, $p = 0.013$). Regarding OS, among cachectic patients classified using the Fearon criteria, a significant association was observed between the *ESR2* rs928554 CC genotype and prolonged OS (Log-rank test, $p = 0.039$). Considering the CASC-IN tool, the *ESR1* rs9340799 A allele was associated with longer OS among non-cachectic patients (Tarone-Ware test, $p = 0.007$).

Conclusion

These findings suggest that oestrogen-related SNPs may serve as potential biomarkers for assessing CAC risk and prognosis, aiding in supportive care interventions. Further investigation is needed to confirm the robustness of these results.

1. Introduction

Cancer remains one of the leading causes of morbidity and mortality worldwide, imposing a substantial clinical and societal burden [1]. In addition to the disease itself, cancer patients often experience multiple complications that negatively impact their clinical progression and quality of life. Among these, cancer-associated cachexia (CAC) is one of the most prevalent and debilitating syndromes [2].

Cachexia is a progressive, multifactorial condition defined by ongoing loss of skeletal muscle mass, with or without a depletion of adipose tissue, that cannot be completely reversed by conventional nutritional support [2, 3]. This syndrome affects over half of oncology patients and is responsible for approximately 20% of cancer-related deaths. Clinically, CAC is associated with marked physical decline, impaired social and functional well-being, reduced tolerance and responsiveness to antineoplastic treatments, increased incidence of postoperative complications, and shortened overall survival (OS) [4]. These consequences highlight CAC as not only a metabolic disorder but also a major determinant of prognosis and therapeutic efficacy in cancer patients.

The aetiology of CAC is highly complex and multifactorial. Disease susceptibility reflects the interplay between patient-related factors (such as advanced age and, albeit controversially, male sex), cancer-related factors (for instance, tumour primary site, with higher CAC prevalence being observed in patients with malignancies of the digestive tract, particularly pancreatic cancer), and treatment-related factors (with chemotherapy and radiotherapy promoting a high pro-cachectic potential) [2, 5]. At the molecular level, the syndrome is driven by dysregulated immune, metabolic and hormonal mechanisms that together lead to muscle wasting and the characteristic metabolic alterations of the syndrome [2].

Regarding hormonal processes, oestrogens appear to play a particularly important role [6, 7]. Beyond their well-established involvement in reproductive physiology, oestrogens contribute to the regulation of multiple pathological conditions, including obesity, osteoporosis, endometriosis, and the pathogenesis of diverse cancer types [8, 9]. The mechanisms of oestrogen action are mediated through two main pathways: genomic (classical) signalling and non-genomic signalling [10]. In the genomic pathway, oestrogens bind to oestrogen receptor alpha (ERα) and beta (ERβ), encoded by the *ESR1* and *ESR2* genes, respectively [11, 12].

In the context of cachexia, oestrogens have been implicated in regulating skeletal muscle homeostasis by enhancing oxidative metabolism and preserving muscle protein integrity, processes directly relevant to preventing muscle wasting. Consistently, oestrogens have been explored as potential therapeutic agents for cancer-associated muscle wasting [13].

Interindividual variability in the development and severity of CAC suggests that genetic predisposition may also contribute to susceptibility. Genetic variants that modulate inflammatory, metabolic, and hormonal responses are increasingly recognised as important determinants of cachexia risk [2, 14]. In this context, variants within *ESR1* and *ESR2* are of particular interest. The *ESR1* polymorphisms rs2046210 (G > A) and rs9340799 (XbaI; A > G), which are characterised as single-nucleotide polymorphisms (SNPs), have been widely studied due to their association with altered ER*α* activity [15]. In *ESR2*, the SNP rs928554 (T > C) appears to modulate ERβ activity, thereby affecting cellular sensitivity to oestrogen [16, 17]. Despite the links between oestrogen signalling and muscle preservation, the potential contribution of genetic variation in *ESR1* and *ESR2* to CAC risk and prognosis remains largely unexplored.

Given the central role of the oestrogen pathway, variants in these genes could represent useful genetic markers for assessing risk and identifying potential therapeutic targets for CAC, thereby supporting earlier preventive interventions in cancer patients at risk. Therefore, this investigation aimed to evaluate the predictive value of SNPs associated with the oestrogen pathway in the development of CAC. Additionally, the prognostic impact of these genetic variants was also examined, with the goal of supporting both earlier risk stratification and personalised preventive strategies.

2. Materials and Methods

2.1. Study Design

A hospital-based, cross-sectional observational study was conducted at the Portuguese Oncology Institute of Porto (IPO Porto), involving adult patients with active cancer attending pre-surgical consultations, the Day Hospital, and the palliative care unit. Ethical approval was obtained from the IPO Porto Ethics Committee (CES IPO: 131/022, differences in disease prevalence), and all participants provided written informed consent before their enrolment.

2.2. Patient Enrolment

Patients were eligible if they were aged 19 years or older and had an Eastern Cooperative Oncology Group Performance Status (ECOG-PS) score of 0 to 3. Exclusion criteria comprised participation in collaborative protocols, attendance solely for a second opinion, receipt of medication specifically for anorexia management, cognitive or consciousness deficits, language barriers, or patient refusal to participate in the study. Between March 2023 and May 2024, 140 patients met the eligibility criteria and were recruited. The mean follow-up time was 45.9 ± 3.2 weeks.

At enrolment, 47% (N = 66) of the patients were > 63 years old and a body mass index (BMI) < 26 kg/m² was observed in 52% (N = 73) of them. Women represented most of the participants (54%; N = 76). Regarding ECOG-PS, 48% (N = 57) of the patients had a score of 0, 33% (N = 39) had a score of 1, 16% (N = 19) had a score of 2, and the remaining participants had a score of 3. Metastatic disease was present in 64% (N = 90) of the cohort, while 6% (N = 8) reported a history of other malignancies. Information on tumour types and treatment modalities is summarised in **Table 1**.

Table 1 - Description of tumour types and oncological treatment approaches in the study cohort (N = 140).

Variable		N (%)
Tumour site	Gastrointestinal	55 (39 %)
	Breast	18 (13 %)
	Lung	16 (11 %)
	Urological	16 (11 %)
	Head and neck	12 (9 %)
	Gynaecological	9 (6 %)
	Haematological	5 (4 %)
	Skin	5 (4 %)
	Sarcoma	3 (2 %)
	Central nervous system	1 (1 %)
Oncological treatment	Chemotherapy	43 (31 %)
	Palliative care	32 (23 %)
	Chemotherapy and immunotherapy	18 (13 %)
	Surgery	15 (11 %)
	Immunotherapy	14 (10 %)
	Chemotherapy and radiotherapy	8 (6 %)
	Hormonal therapy and immunotherapy	5 (4 %)
	Hormonal therapy	3 (2 %)
	Chemotherapy and hormonal therapy	2 (1 %)

Abbreviation: N, number of participants.

2.3. Diagnostic Tools

The Fearon criteria and the Cachexia Scoring Tool (CASC-IN) were employed for cachexia diagnosis. Although no universal consensus exists, the Fearon criteria are the most extensively studied and classify patients as non-cachectic, pre-cachectic, cachectic, or refractory cachectic [18, 19]. In comparison with the Fearon criteria, the CASC-IN score provides a more comprehensive assessment of inflammation and metabolic alterations [20, 21].

For both instruments, the diagnosis of CAC was established by an experienced dietitian on the same day as patient recruitment.

2.4. Selection of Genetic Variants

Polymorphism selection was based on their relevance in modulating the oestrogen pathway. In addition, their functional consequences and minor allele frequency (MAF) were considered, based on data from the Ensembl platform (https://www.ensembl.org/index.html, last accessed on 9th February 2026). Specifically, only genetic variants with an MAF greater than 15% were included to ensure representation of all SNP genotypes within the study population. Finally, the availability of TaqMan® probes designed for allelic discrimination of the identified SNPs (Thermo Fisher Scientific, Waltham, MA, USA) was also taken into account. Applying these criteria, the *ESR1* polymorphisms rs2046210 (G>A) and rs9340799 (A>G), and *ESR2* rs928554 (T>C), were deemed the most suitable variants to be evaluated.

2.5. Sample Collection and Genomic DNA Extraction

At the time of patient recruitment (same day of CAC diagnosis), peripheral blood samples were collected from each participant at the phlebotomy unit of IPO Porto using a standard venepuncture method. Samples were drawn into tubes containing ethylenediaminetetraacetic acid (EDTA).

For genomic DNA extraction, a portion of each venous blood sample was labelled with an internal research group code to ensure data protection and subsequently stored at -20ºC. Genomic DNA was extracted using the MagaBio Plus Virus DNA/RNA Purification Kit II (BSC71S1E, Bioflux®, Japan) in combination with the MGISP-NE32 automated nucleic acid extraction system (MGI Tech), following the supplier's instructions. The purity and yield of the isolated DNA were evaluated using a NanoDrop Lite instrument (Thermo Fisher Scientific, Waltham, MA, USA). The extracted DNA samples were subsequently stored at -20 °C until further analysis.

2.6. Polymorphism Genotyping

Genotyping of the selected SNPs was performed by real-time polymerase chain reaction (qPCR) using a StepOnePlus™ qPCR system (Applied Biosystems®, Carlsbad, CA, USA). A TaqMan probe-based approach was employed, with specific genotyping assays designed for each SNP (**Supplementary Table S1**).

Each PCR reaction well contained a final volume of 6 µL, comprising 2.5 µL of TaqPath™ ProAmp™ Master Mix (1×), 2.375 µL of nuclease-free water, 0.125 µL of the TaqMan® SNP Genotyping Assay (Applied Biosystems®, Carlsbad, CA, USA), and 1.0 µL of genomic DNA from each participant. Negative controls (lacking template DNA) were included on all PCR plates to assess potential contamination and false-positive amplification. For quality assurance, 10% of the samples were randomly selected and genotyped to verify the accuracy and reproducibility of the results. Amplification data were analysed using StepOne™ software, version 2.3 (Applied Biosystems®, Carlsbad, CA, USA).

The interpretation of genotyping results was conducted independently by two researchers, who were blinded to the patients' demographic and clinicopathological data.

2.7. Statistical analysis

Statistical analyses were carried out using IBM SPSS Statistics for Windows (version 30.0; IBM Corp., Armonk, NY, USA).

Genotype distributions observed in the study population were compared with reference frequencies for the Iberian population obtained from the Ensembl database (https://www.ensembl.org/index.html, last accessed on 9th February 2026). Agreement between observed and expected genotype proportions was evaluated by testing for Hardy-Weinberg equilibrium (HWE) using the chi-square (χ^2) test.

Given the cohort size (N > 50), the normality of continuous variables was assessed with the Kolmogorov-Smirnov test. Variables demonstrating a parametric distribution were described using mean values, whereas for non-parametric variables, medians were used.

For both the Fearon criteria and the CASC-IN tool, CAC status was analysed using a three-level categorisation (non-cachectic, pre-cachectic and cachectic) and a binary grouping, in which pre-cachectic and cachectic patients were combined into a single entity.

Associations between the SNPs and the presence of CAC, as well as demographic and clinicopathological characteristics, were examined using the χ^2 test or Fisher's exact test, depending on expected cell counts. Logistic regression models were applied to estimate disease risk associated with each SNP genotype, expressed as odds ratios (ORs) with corresponding 95% confidence intervals (95% CIs).

OS was selected as the primary clinical endpoint and defined as the interval from study enrolment (coinciding with CAC assessment) to death from any cause or the date of last follow-up for censored participants. Survival analyses were performed using the Kaplan-Meier method. Differences between groups were assessed using the Log-rank test or Tarone-Ware test, as appropriate, based on proportional hazards assumptions and the inheritance model suggested by initial inspection of survival curves. Cox proportional hazards regression was employed to evaluate the effect of the SNPs on mortality risk. Multivariable models were adjusted for relevant demographic and clinicopathological covariates, namely patients' age, sex, BMI and metastatic status at the time of patient recruitment (with vs. without). Notably, due to cohort size and, consequently, the limited statistical power, stratified analyses according to tumour type and cancer treatment approaches were not conducted. To assess the robustness of the findings, internal validation was conducted using bootstrap resampling with 1,000 iterations.

All statistical tests were two-sided, and statistical significance was defined as $p < 0.05$.

3. Results

3.1. Allelic and Genotypic Frequencies of the Polymorphisms

The allelic and genotypic distribution of the SNPs in the study cohort is shown in **Table 2**. All variants were found to be in HWE (χ^2 test, $p > 0.05$), indicating no significant difference in genotype distribution in the study cohort compared with that reported for the Iberian population.

Table 2 - Allelic and genotypic distribution of the SNPs.

SNP	Genomic location	Genotype	MAF Iberian population (%) (MA)	Distribution Iberian population* (%)	MAF Study cohort (%) (MA)	Distribution Study cohort N (%)
***ESR1* rs2046210**	6q25.1	AA	40 (A)	17	40 (A)	17 (12)
		GG		37		46 (33)
		AG		46		77 (55)
***ESR1* rs9340799**	6q25.1	AA	34 (G)	42	34 (G)	61 (44)
		GG		10		16 (11)
		AG		48		63 (45)
***ESR2* rs928554**	14q23.3	CC	43 (C)	16	40 (C)	21 (15)
		TT		31		50 (36)
		CT		53		69 (49)

*According to data from the Ensembl platform (https://www.ensembl.org/index.html, last accessed on 9th February 2026). Abbreviations: SNP, single-nucleotide polymorphisms; MA, minor allele; MAF, minor allele frequency; N, number of patients.

3.2. Association between the Polymorphisms and Clinicopathological Factors

Associations between the SNPs and patients' age, sex, BMI and metastatic status at the time of patient recruitment were analysed. No significant associations were observed for *ESR1* rs2046210, *ESR1* rs9340799 and *ESR2* rs928554 (χ^2 test and Fisher's exact test, $p > 0.05$), meaning the allelic and genotypic distribution of the SNPs is independent of these factors.

3.3. Impact of the Polymorphisms on CAC Status

For the *ESR1* rs9340799 and *ESR2* rs928554 SNPs, no association with CAC status was observed, either in the overall cohort or in stratified analyses (χ^2 test and Fisher's exact test, $p > 0.05$). Regarding *ESR1* rs2046210, although no significant association was identified in the total cohort, sex-stratified analysis revealed a statistically significant association in the male subgroup, according to the Fearon criteria

(with CAC vs. without CAC). Specifically, those carrying the AA genotype exhibited a 49% protective effect compared with G allele carriers (AA vs. GA/GG; OR = 0.49; CI 95%: 0.38-0.54; Fisher's exact test, p = 0.013; **Table 3**). In the female group, no significant association was observed (Fisher's exact test, p = 1.000; **Table 3**). Considering the CASC-IN tool, no statistically significant association was detected (Fisher's exact test, $p > 0.05$). Likewise, with respect to other stratified analyses (age, BMI and metastatic status), no significant finding was observed (Fisher's exact test, $p > 0.05$).

Table 3 - Distribution of *ESR1* rs2046210 genotypes by CAC status and patient sex.

	***ESR1* rs2046210 genotype**	**Fearon Classification**		**Fisher's exact test *p*-Value**	**Crude OR**	**CI 95%**
		Without CAC N (%)	**With CAC N (%)**			
Females (N = 76)	AA	6 (60)	4 (40)	1.000	-	-
	AG/GG	42 (64)	24 (36)		-	-
Males (N = 64)	AA	7 (100)	0 (0)	**0.013**	0.49	**0.38-0.64**
	AG/GG	28 (49)	29 (51)		Reference	-

Bold values were classified as statistically significant. Abbreviations: CAC, Cancer-associated cachexia; CI, confidence interval; OR, odds ratio; N, number of patients included in the analysis.

3.4. Effect of the Polymorphisms on Patient Survival

Considering the entire cohort (N = 140), no significant impact of the SNPs on OS was observed (Log-rank and Tarone-Ware tests, $p > 0.05$). Subgroup analyses were subsequently performed according to CAC status, defined using the Fearon criteria and the CASC-IN tool.

When stratified using the Fearon criteria, no significant association was found between *ESR1* rs2046210 and patient OS in either subgroup, irrespective of the genetic model applied (Log-rank and Tarone-Ware test, $p > 0.05$). Nevertheless, although the difference did not reach statistical significance (Tarone-Ware test, p = 0.087; **Fig. 1a**), carriers of the A allele exhibited a notably longer mean OS (41.8 ± 4.3 weeks) compared with individuals with the GG genotype (20.7 ± 2.6 weeks). No significant association concerning this SNP and patient survival was observed in the univariate and multivariate Cox analyses, regardless of genetic model and CAC status ($p > 0.05$).

For *ESR2* rs928554, stratification by CAC status according to the Fearon criteria revealed a significant association exclusively within the cachectic subgroup (Log-rank test, p = 0.039). In this group, the CC genotype appeared to confer a survival advantage relative to carriers of the T allele. However, interpretation is limited by the small number of individuals with the CC genotype and the fact that all

such cases were censored, precluding estimation of mean OS across genotypes. No significant association was observed in the univariate Cox analysis, irrespective of the genetic model applied or CAC status ($p > 0.05$). In contrast, the multivariate Cox analysis identified a significant association, but only in the non-cachectic subgroup (**Table 4**). In this analysis, the CC genotype was associated with an increased risk of death, in contrast to the apparent protective effect observed in the cachectic subgroup.

Table 4. Multivariable Cox analysis on the risk of death among non-cachectic patients

Variable	aHR	95%CI	*p*-Value	*Bootstrap p-Value*
Fearon criteria (N = 83)				
ESR2 rs928554 (CC vs. TT/TC *)	11.0	**1.0-116.9**	**0.047**	**0.001**
Patient sex (female vs. male *)	1.53	0.2-11.6	0.683	0.486
Patient age at enrolment (≥ 63 vs. < 63 years *)	2.17	0.2-24.5	0.531	0.341
BMI at recruitment (< 26 vs. ≥ 26 kg/m^2 *)	2.23	0.4-13.0	0.374	0.273
Metastatic status (with vs. without *)	5.31	0.5-56.3	0.166	**0.012**
CASC-IN tool (N = 88)				
ESR1 rs9340799 (GG vs. AA/AG *)	6.9	**1.5-32.2**	**0.013**	**0.002**
Patient sex (female vs. male *)	0.6	0.1-2.6	0.462	0.466
Patient age at enrolment (≥ 63 vs. < 63 years *)	1.9	0.4-9.0	0.434	0.345
BMI at recruitment (< 26 vs. ≥ 26 kg/m^2 *)	1.8	0.4-7.3	0.410	0.333
Metastatic status (with vs. without *)	1.3	0.3-4.8	0.710	0.721

Bold values were deemed statistically significant. * - reference group. Abbreviations: aHR, adjusted hazard ratio; BMI, body mass index; CI, confidence interval.

Finally, no significant association between *ESR1* rs9340799 and patient survival was observed in the subgroup analysis based on the Fearon criteria.

Using the CASC-IN tool for stratification, a significant association between *ESR1* rs9340799 and OS was identified among non-cachectic patients (Tarone-Ware test, $p = 0.007$; **Fig. 2a**). Namely, carriers of the A allele genotypes demonstrated longer survival time than those carrying the GG genotype (mean OS = 45.0 ± 4.3 weeks and 26.9 ± 3.0 weeks, respectively). Consistently, in this subgroup, patients harbouring the GG genotype showed a fivefold higher risk of death compared with A allele carriers [hazard ratio (HR) = 5.0; 95% CI, 1.2-20.9; $p = 0.029$]. Importantly, this association remained statistically significant after adjustment for patient sex, age, BMI and metastatic status, supporting the independent prognostic value

of *ESR1* rs9340799 (**Table 4**). In opposition, no significant association was detected among cachectic patients (Tarone-Ware test, $p = 0.492$; **Fig. 2b**). Regarding the other SNPs, no significant association was observed, irrespective of the genetic model applied (Log-rank and Tarone-Ware tests, $p > 0.05$).

4. Discussion

The development of CAC arises from the intricate crosstalk between inflammatory, metabolic and endocrine mechanisms [2, 5]. Within this framework, oestrogen signalling, acting through ERα and ERβ, encoded by *ESR1* and *ESR2* respectively, has been implicated in the regulation of skeletal muscle integrity and protein turnover [6, 7, 11-13]. While *ESR1* rs2046210 and rs9340799, and *ESR2* rs928554 have been linked to variations in receptor function and cancer-related clinical outcomes, their role in influencing susceptibility to, or prognosis of, CAC remains poorly explored [15]. Hence, this study investigated whether genetic variation within the oestrogen signalling axis could act as a predictive and prognostic indicator for CAC, thereby contributing to improved disease risk assessment and more individualised preventive approaches.

The rs2046210 polymorphism (G > A) is an intergenic variant located at chromosome 6q25.1, near the *ESR1* gene. It involves the substitution of a guanine (G) base with adenine (A) [22, 23]. Although it does not alter the coding sequence of *ESR1*, this SNP has been implicated in the regulation of the gene expression. Evidence from non-malignant tissues indicates that the A allele is associated with increased *ESR1* transcription, whereas this relationship appears to be attenuated or absent in tumour samples, suggesting tissue-specific regulatory effects [24]. To date, most investigations of rs2046210 have focused on breast cancer and endometriosis, consistently reporting the A allele as a susceptibility variant for these conditions [15, 22, 25-27].

In the present study, while no significant relationship was detected in the overall cohort (N = 140), a statistically significant association between *ESR1* rs2046210 and CAC status (according to Fearon criteria) emerged exclusively among male participants. Specifically, those with the AA genotype demonstrated an approximately 49% lower likelihood of developing CAC compared with the G allele carriers (Fisher's exact test, $p = 0.013$). This sex-specific finding is biologically plausible. While oestrogens are traditionally linked to female physiology, with their circulating levels being approximately twice as high in women, they also play important regulatory roles in males, where the majority (around 80%) of oestrogens are derived from peripheral aromatisation of testosterone [28]. Importantly, sexual dimorphism in CAC has been reported, with male sex generally described as a risk factor for disease development [2]. Differences in circulating sex hormone levels and receptor-mediated signalling may therefore partly underlie these disparities. From a mechanistic perspective, oestrogens, acting through ERα, have been shown to attenuate pro-inflammatory signalling and modulate key regulators of muscle mass, thus exerting protective effects on skeletal muscle homeostasis [29]. Overall, the evidence suggests that genetic variation in *ESR1* may exert differential effects depending on the hormonal milieu. Considering both the literature and the findings of the present study, enhanced oestrogen receptor signalling in male carriers of the AA genotype could hypothetically mitigate inflammatory and catabolic

pathways implicated in muscle wasting, offering a potential explanation for the observed protective association. Nonetheless, given the limited sample size and the absence of functional data in the present study, this interpretation should be considered exploratory and warrants validation in larger, independent cohorts.

Concerning survival outcomes, *ESR1* rs2046210 was not significantly associated with OS in the full cohort. Similarly, no significant relationship was identified after stratification by CAC status under any genetic model (Log-rank and Tarone-Ware tests; $p > 0.05$). Although a non-significant trend towards improved survival was observed among A allele carriers, who demonstrated a longer mean survival time than their counterparts (Tarone-Ware test, $p = 0.087$), this difference did not reach statistical significance. The lack of significance in both univariate and multivariate Cox regression analyses ($p > 0.05$), irrespective of genetic model and CAC status, may reflect limited statistical power, potential confounding by clinical variables, and the multifactorial nature of survival in cancer patients. In this framework, while the survival trend is directionally consistent with a possible protective role of the A allele for CAC development, these findings should be interpreted with caution and confirmed in larger studies designed to robustly assess prognostic effects.

The rs9340799 (Xbal) polymorphism (A > G) is an intronic variant within the *ESR1* gene (chromosome 6q25.1) and, like other intronic SNPs, is hypothesised to influence gene expression rather than protein structure directly [23, 30]. While the precise molecular mechanism remains unresolved, some studies have suggested that rs9340799 may affect transcription factor binding and messenger RNA stability, potentially altering *ESR1* expression in a context-dependent manner [31]. Similar to *ESR1* rs2046210, rs9340799 has been studied in the context of breast cancer, but with inconsistent results. One study demonstrated that carriers of the variant G allele (GG and AG genotypes) had a significantly reduced risk of developing the disease [12]. However, a large meta-analysis found no consistent association between the SNP and breast cancer susceptibility across multiple genetic models, either in the entire cohort or in subgroup analyses by ethnicity and menopausal status, suggesting that this genetic variant is unlikely to be a strong independent marker for breast cancer risk [32]. Beyond malignant diseases, there is scarce data on the association of *ESR1* rs9340799 with metabolic traits. For example, the SNP G allele has been associated with reduced waist circumference, suggesting a role in regulating abdominal fat distribution [33]. In postmenopausal women, carriers of the G allele also exhibited lower BMI and reduced body fat percentage [31]. Additionally, the rs9340799 G allele has been previously associated with type 2 diabetes mellitus in Caucasian populations. However, more recent evidence indicates no clear association with either increased or decreased disease risk [34].

In the context of cachexia, no direct associations with *ESR1* rs9340799 have been established. In the present study, no significant association between the SNP and CAC status was observed, irrespective of genetic model, diagnostic criterion or subgroup analyses (χ^2 test and Fisher's exact test, $p > 0.05$). Regarding patient survival, the SNP was not significantly associated with OS in the entire cohort or when stratified according to the Fearon criteria. In contrast, using the CASC-IN tool, a significant association between *ESR1* rs9340799 and patient survival was identified in non-cachectic patients (Tarone-Ware

test, $p = 0.007$). In this subgroup, carriers of the A allele genotypes had longer survival time than those carrying the GG genotype, and the latter showed a fivefold higher risk of death ($p = 0.029$). Importantly, this association remained statistically significant after adjustment for patient sex, age, BMI and metastatic status, supporting the independent prognostic value of *ESR1* rs9340799 in this specific clinical context. In opposition, no significant association was detected among cachectic patients (Tarone-Ware test, $p = 0.492$). Given the lack of clear functional data for the variant, these findings require replication in larger cohorts and functional studies to elucidate underlying mechanisms.

The rs928554 polymorphism (T > C) is located in the 3' untranslated region (3' UTR) of the *ESR2* gene (chromosome 14q23.2) [17, 23]. Given its location, the SNP is hypothesised to influence gene expression. Yet, in a previous study, no significant differences were observed in ERb mRNA stability or translational efficiency between alleles. Overall, this polymorphism remains poorly characterised in the scientific literature [17]. Similar to the aforementioned *ESR1* SNPs, *ESR2* rs928554 has been investigated in relation to breast cancer. Notably, one study reported that the A allele is associated with a reduced risk of triple-negative breast cancer [35]. Furthermore, this SNP has been linked to eating disorders such as bulimia nervosa [36]. Regarding cachexia, to the best of our knowledge, no direct associations have been reported.

Similar to *ESR1* rs9340799, *ESR2* rs928554 was not associated with CAC status across all genetic models, CAC diagnostic tools and subgroup analyses (χ^2 and Fisher's exact tests, $p > 0.05$). Regarding patient survival, a significant association between *ESR2* rs928554 and OS was detected only in subgroup analyses stratified by CAC status according to the Fearon criteria. In the cachectic group, the CC genotype appeared to confer a survival advantage relative to carriers of the T allele subgroup (Log-rank test, $p = 0.039$). In the univariate Cox analysis, no significant association was observed ($p > 0.05$). In contrast, in the multivariate Cox analysis, a significant association was observed, but only in the subgroup of non-cachectic patients, with CC being associated with an increased risk of patient death. These divergent associations may reflect context-dependent effects of ERβ signalling under differing systemic conditions. ERβ is known to regulate pathways involved in inflammation, metabolism and cellular proliferation, processes that are central to both tumour progression and CAC pathophysiology [13]. In the cachectic state, where systemic inflammation and metabolic dysregulation predominate, variation in *ESR2* may influence host-tumour interactions differently than in patients without CAC, potentially accounting for the contrasting survival patterns observed. However, these findings must be interpreted with caution, given the small number of individuals with the CC genotype in the cachectic subgroup and the absence of events within this group, which limits the robustness of the survival estimates.

No significant association was observed between *ESR2* rs928554 and patient survival when conducting the subgroup analysis using the CASC-IN tool, regardless of the genetic model applied (Log-rank and Tarone-Ware tests, $p > 0.05$). Given these inconsistencies and the absence of clear functional evidence, the clinical significance of the SNP remains uncertain and merits further comprehensive investigation [17].

Taken together, this study provides preliminary evidence that genetic variation within the oestrogen signalling pathway may influence both susceptibility to CAC and survival differences among cancer patients. The identification of such genetic biomarkers holds potential clinical significance, offering opportunities for improved risk stratification, earlier interventions, and more personalised supportive and therapeutic strategies. Integrating genetic information into clinical decision-making could help identify patients at higher risk of adverse outcomes, who may benefit from closer monitoring, nutritional optimisation, or targeted supportive care.

Despite these promising findings, they should be interpreted with caution due to the study's limitations. The single-centre design and relatively modest sample size may have limited statistical power and reduce the generalisability of the results. Furthermore, the absence of robust functional data prevented direct assessment of genotype-phenotype correlations. Accordingly, replication and validation of these findings in larger, multicentre, and ethnically diverse cohorts are necessary. Future studies should aim to integrate genetic data with metabolic, inflammatory, hormonal, and clinical parameters to provide a more comprehensive understanding of how *ESR1* and *ESR2* polymorphisms influence CAC pathogenesis. Such research will be critical for elucidating the mechanistic and clinical significance of these variants and for evaluating the feasibility of genotype-guided preventive or therapeutic interventions. Additionally, investigating other functionally relevant variants in *ESR1* and *ESR2* may further clarify how inherited genetic differences in oestrogen metabolism modulate muscle homeostasis, cachexia development, and patient survival.

5. Conclusion

Beyond environmental and disease-related factors, growing evidence indicates that host genetic susceptibility influences CAC risk and clinical trajectories. Given the involvement of hormonal and inflammatory pathways in the regulation of metabolism, body composition, and muscle homeostasis, polymorphisms within oestrogen receptor genes emerge as biologically plausible contributors to CAC pathogenesis. In this context, selected SNPs in *ESR1* and *ESR2* were evaluated as potential biomarkers. No statistically significant associations were detected between the analysed variants and CAC status in the overall cohort. However, sex-stratified analysis revealed a significant association in male patients for *ESR1* rs2046210 (G>A), in which the AA genotype demonstrated to be protective against the disease development. These findings indicate that the influence of oestrogen receptor polymorphisms may be sex-specific, as expected. Beyond CAC susceptibility, the findings further suggest that variation in *ESR1* and *ESR2* may affect survival outcomes depending on CAC status. Taken together, these findings provide preliminary evidence that oestrogen-related genetic variation may contribute to both CAC risk and patient outcomes, supporting their potential role as biomarkers in clinical settings. While these results require validation in larger, independent cohorts, they open avenues for more personalised approaches to cachexia management. Integrating genetic markers into clinical assessment tools could enhance early identification of at-risk patients, inform supportive care strategies, and ultimately contribute to improving both survival and quality of life in individuals affected by CAC.

Declarations

Acknowledgements: The authors would like to thank Ministério da Saúde de Portugal, Portuguese Institute of Oncology of Porto (IPO Porto), Portuguese League Against Cancer - Northern Branch (LPCC-NRN), Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), Institute for Innovation, Capacity Building and Sustainability of Agri-Food Production (Inov4Agro) and Fundação para a Ciência e Tecnologia (FCT).

Funding: This work was supported by the Portuguese Institute of Oncology of Porto (IPO Porto) (PI127-CI-IPOP-118-2019), Centre for the Research and Technology of Agro-Environmental and Biological Science (CITAB) [UIDB/04033/2025 (DOI: 10.54499/UIDB/04033/2025] and Institute for Innovation, Capacity Building and Sustainability of Agri-Food Production (Inov4Agro) [LA/P/0126/2020 (DOI: 10.54499/LA/P/0126/2020)] and Fundação para a Ciência e Tecnologia (FCT). During the investigation and manuscript writing, T.F., I.G.d.M. and V.T. were research fellowship holders supported by the Portuguese League Against Cancer - Northern Branch (LPCC-NRN). J.M.O.S. also contributed to this study as an LPCC-NRN 2023 Research Fellow. The institutions did not influence the writing and publishing of this article.

Competing Interests: The authors declare no conflicts of interest.

Author contribution: Conceptualisation, B.G.S. and V.T.; formal analysis, B.G.S., R.M. and V.T.; Methodology, B.G.S., A.C.L.S. and V.Z. and J.M.O.S.; investigation, B.G.S., A.C.L.S., T.F., C.S., V.R.D. and M.P.S and J.M.O.S.; resources, R.M.; writing-original draft preparation, B.G.S.; writing-review and editing, B.G.S., A.C.L.S., V.Z., C.S., V.R.D., M.P.S., T.F., I.G.d.M., J.M.O.S., R.M. and V.T.; supervision, I.G.d.M, J. M.O.S., R.M. and V.T; funding acquisition, T.F., I.G.d.M., J.M.O.S., R.M. and V.T. All authors have read and agreed to the published version of the manuscript.

Ethics approval: Approval was granted by the Human Research Ethics Committee of IPO Porto (CES. IPO 131/022, approved on 28th July 2022).

Consent to Participate: Informed consent, in line with the principles of the Declaration of Helsinki, was obtained from all individual participants included in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

References

1. Bray, F., et al., *Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries*. CA: a cancer journal for clinicians, 2024. 74(3): p. 229–263.

2. Liz-Pimenta, J., et al., *Thrombosis and cachexia in cancer: Two partners in crime?* Critical Reviews in Oncology/Hematology, 2023. 186: p. 103989.
3. Tisdale, M.J., *Mechanisms of cancer cachexia*. Physiological reviews, 2009. 89(2): p. 381–410.
4. Onesti, J.K. and D.C. Guttridge, *Inflammation based regulation of cancer cachexia*. BioMed research international, 2014. 2014(1): p. 168407.
5. Yoo, W., et al., *Pancreatic cancer induces muscle wasting by promoting the release of pancreatic adenocarcinoma upregulated factor*. Experimental & Molecular Medicine, 2021. 53(3): p. 432–445.
6. Norman, A.W. and G. Litwack, *Hormones*. 1997: Academic press.
7. Hiller-Sturmhöfel, S. and A. Bartke, *The endocrine system: an overview*. Alcohol health and research world, 1998. 22(3): p. 153.
8. Halbe, H.W., *Biossíntese dos estrogênios*. Revista de Medicina, 1965. 49(4): p. 226–234.
9. Hamilton, K.J., et al., *Estrogen hormone biology*. Current topics in developmental biology, 2017. 125: p. 109–146.
10. Björnström, L. and M. Sjöberg, *Mechanisms of estrogen receptor signaling: convergence of genomic and nongenomic actions on target genes*. Molecular endocrinology, 2005. 19(4): p. 833–842.
11. Chen, P., B. Li, and L. Ou-Yang, *Role of estrogen receptors in health and disease*. Frontiers in endocrinology, 2022. 13: p. 839005.
12. Leyland-Jones, B., et al., *ESR1 and ESR2 polymorphisms in the BIG 1–98 trial comparing adjuvant letrozole versus tamoxifen or their sequence for early breast cancer*. Breast cancer research and treatment, 2015. 154: p. 543–555.
13. Counts, B.R., et al., *The effect of estradiol administration on muscle mass loss and cachexia progression in female Apc Min/+ mice*. Frontiers in Endocrinology, 2019. 10: p. 720.
14. Johns, N., et al., *Genetic basis of interindividual susceptibility to cancer cachexia: selection of potential candidate gene polymorphisms for association studies*. Journal of genetics, 2014. 93: p. 893–916.
15. Proestling, K., et al., *The rs2046210 Polymorphism Is Associated with Endometriosis Risk and Elevated Estrogen Receptor 1 Expression in the Eutopic Endometrium of Women with the Disease*. Biomedicines, 2024. 12(8): p. 1657.
16. Szaflik, T., et al., *Polymorphisms in the 3' UTR Region of ESR2 and CYP19A1 Genes in Women with Endometriosis*. European Journal of Obstetrics & Gynecology and Reproductive Biology, 2020. 250: p. 241–245.
17. Putnik, M., et al., *Effects of two common polymorphisms in the 3'untranslated regions of estrogen receptor β on mRNA stability and translatability*. BMC genetics, 2009. 10: p. 1–11.
18. Fearon, K., et al., *Definition and classification of cancer cachexia: an international consensus*. The lancet oncology, 2011. 12(5): p. 489–495.
19. Huo, Z., et al., *Diagnostic Criteria for Cancer-Associated Cachexia: Insights from a Multicentre Cohort Study*. Journal of Cachexia, Sarcopenia and Muscle, 2025. 16(1): p. e13703.

20. Argilés, J., et al., *CASC-IN: a new tool to diagnose pre-cachexia in cancer patients.* Ann Clin Oncol. https://doi.org/10.31487/j. ACO, 2019. 3.
21. Permuth, J.B., et al., *Leveraging real-world data to predict cancer cachexia stage, quality of life, and survival in a racially and ethnically diverse multi-institutional cohort of treatment-naïve patients with pancreatic ductal adenocarcinoma*. Frontiers in oncology, 2024. 14: p. 1362244.
22. Miedl, H., et al., *Association of the estrogen receptor 1 polymorphisms rs2046210 and rs9383590 with the risk, age at onset and prognosis of breast cancer*. Cells, 2023. 12(4): p. 515.
23. Harrison, P.W., et al., *Ensembl 2024*. Nucleic acids research, 2024. 52(D1): p. D891-D899.
24. Sun, Y., et al., *Evaluation of potential regulatory function of breast cancer risk locus at 6q25. 1*. Carcinogenesis, 2016. 37(2): p. 163–168.
25. Guo, H., et al., *A common polymorphism near the ESR1 gene is associated with risk of breast cancer: evidence from a case-control study and a meta-analysis*. PloS one, 2012. 7(12): p. e52445.
26. Yang, Z., et al., *Association between a novel polymorphism (rs2046210) of the 6q25. 1 locus and breast cancer risk*. Breast cancer research and treatment, 2013. 139(1): p. 267–275.
27. Thanh, N.T.N., et al., *Two polymorphisms, rs2046210 and rs3803662, are associated with breast cancer risk in a Vietnamese case-control cohort*. Genes & Genetic Systems, 2018. 93(3): p. 101–109.
28. Mauvais-Jarvis, F. and S.H. Lindsey, *Metabolic benefits afforded by estradiol and testosterone in both sexes: clinical considerations*. The Journal of Clinical Investigation, 2024. 134(17).
29. Pellegrino, A., P.M. Tiidus, and R. Vandenboom, *Mechanisms of estrogen influence on skeletal muscle: mass, regeneration, and mitochondrial function*. Sports medicine, 2022. 52(12): p. 2853–2869.
30. Chen, S., et al., *Association between the ESR1-351A > G single nucleotide polymorphism (rs9340799) and adolescent idiopathic scoliosis: a systematic review and meta-analysis*. European Spine Journal, 2014. 23: p. 2586–2593.
31. Montazeri-Najafabady, N. and M.H. Dabbaghmanesh, *Association of estrogen receptor alpha gene PvuII and XbaI polymorphisms with obesity-related phenotypes and body composition in Iranian children and adolescents: a cross-sectional study*. Egyptian Journal of Medical Human Genetics, 2025. 26(1): p. 48.
32. Tan, S., et al., *The rs9340799 polymorphism of the estrogen receptor alpha (ESR1) gene and its association with breast cancer susceptibility. Sci Rep.* 2021; *11*: *18619*.
33. Fox, C.S., et al., *Sex-specific association between estrogen receptor-α gene variation and measures of adiposity: The Framingham Heart Study*. The Journal of Clinical Endocrinology & Metabolism, 2005. 90(11): p. 6257–6262.
34. Ulhaq, Z.S., *Update on "associations of estrogen receptor alpha gene polymorphisms with type 2 diabetes mellitus and metabolic syndrome: a systematic review and meta-analysis"*. Hormone and Metabolic Research, 2020. 52(01): p. 67–70.
35. Sghaier, I., et al., *Unique ESR1 and ESR2 estrogen receptor gene variants associated with altered risk of triple-negative breast cancer: A case-control study*. Gene, 2023. 851: p. 146969.

36. Nilsson, M., et al., *Association of estrogen receptor β gene polymorphisms with bulimic disease in women*. Molecular psychiatry, 2004. 9(1): p. 28–34.

Figures

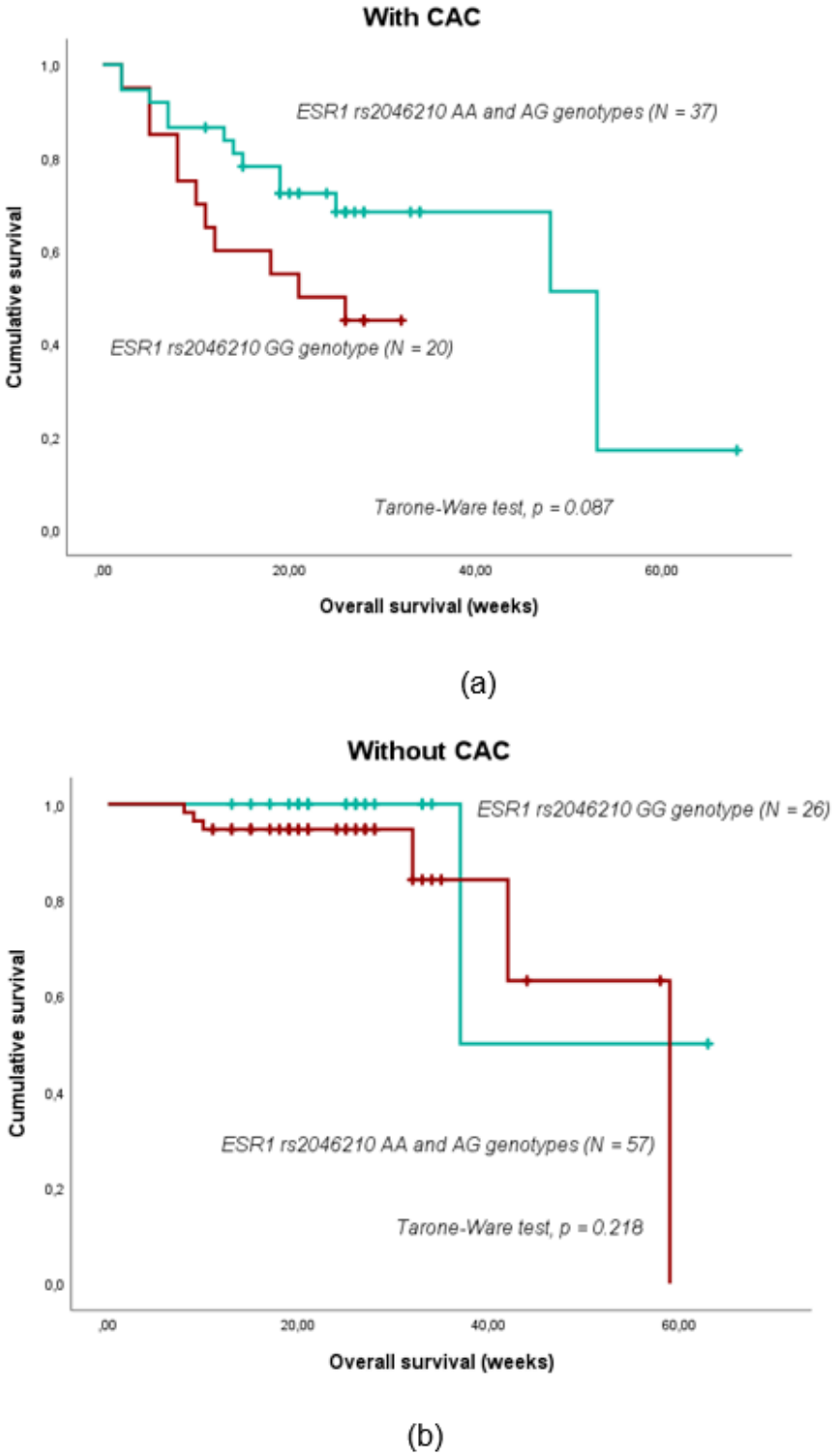
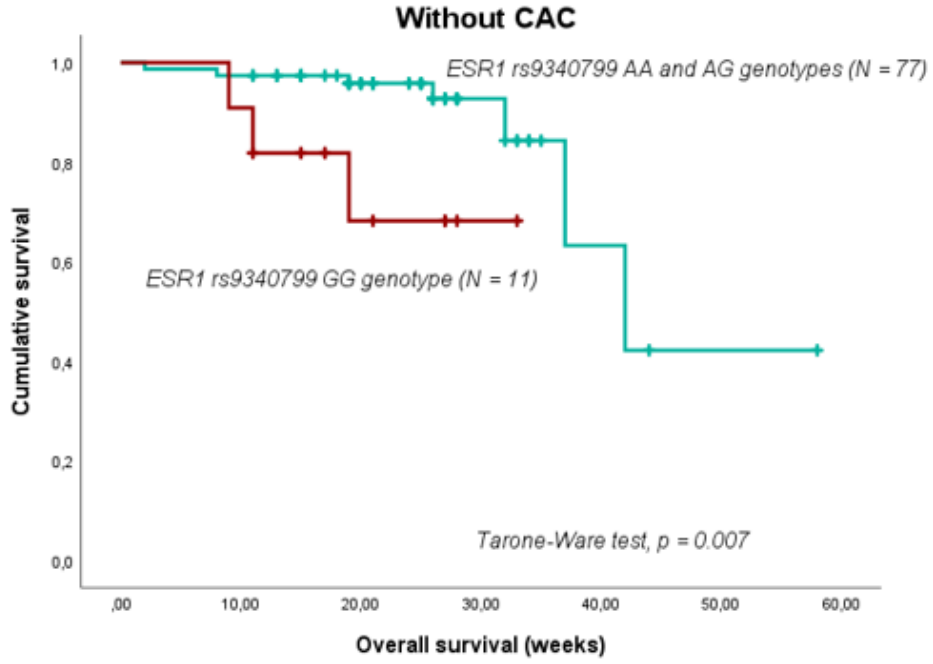
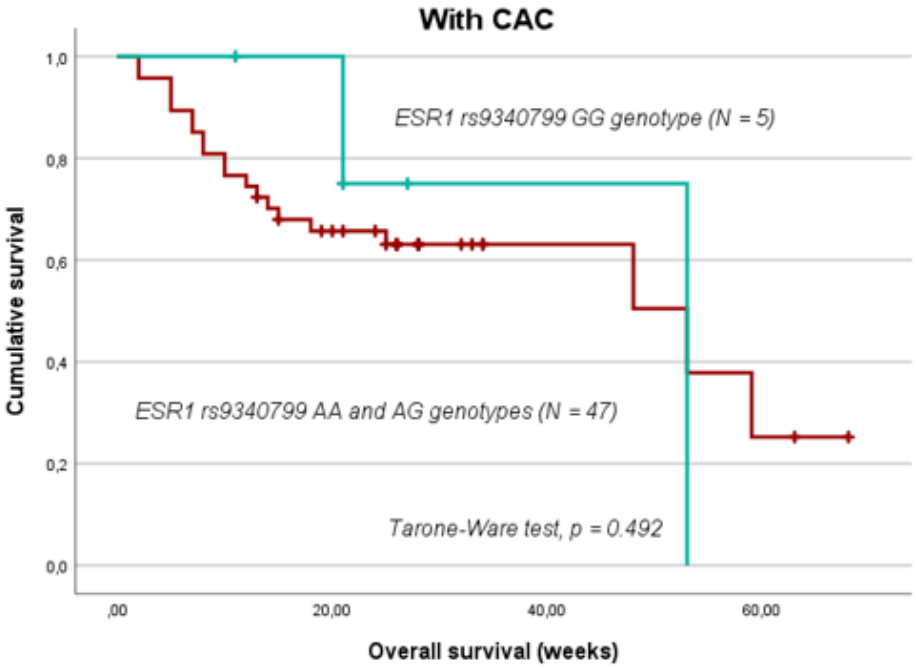


Figure 1

Overall survival (OS) according to *ESR1* rs2046210 genotypes (AA/AG vs. GG), analysed using the Kaplan-Meier method and Tarone-Ware test. **a)** In the subgroup with cancer-associated cachexia (CAC), classified according to the Fearon criteria, although the association did not reach statistical significance, carriers of the A allele showed longer survival than patients with the GG genotype (mean OS = 41.8 ± 4.3 weeks and 20.7 ± 2.6 weeks, respectively; Tarone-Ware test, $p = 0.087$). **b)** In the subgroup without the condition, no difference in survival time was observed between A allele carriers and GG genotype carriers (mean OS = 49.9 ± 4.4 weeks and 50.0 ± 9.2 weeks, respectively; Tarone-Ware test, $p = 0.218$).



(a)



(b)

Figure 2

Overall survival (OS) according to *ESR1* rs9340799 genotypes (AA/AG vs. GG), analysed using the Kaplan-Meier method and Tarone-Ware test. **a)** In the subgroup without cancer-associated cachexia (CAC), classified using the CASC-IN tool, patients with the A allele had higher survival time compared to GG genotype carriers (mean OS = 45.0 ± 4.3 weeks and 26.9 ± 3.0 weeks, respectively; Tarone-Ware test, $p = 0.007$). **b)** In the subgroup with the condition, no significant difference was observed comparing the survival time of A allele genotypes and GG genotype carriers (mean OS = 41.0 ± 4.2 weeks and 45.0 ± 9.8 weeks, respectively; Tarone-Ware test, $p = 0.492$).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryfile.xlsx