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Exploratory Associations of *ESR1* Variants with Biomarker and Tolerability Outcomes in Iraqi Postmenopausal Women Receiving Letrozole for Estrogen Receptor-Positive Breast Cancer

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ABSTRACT

Background & Objective: Letrozole is a standard aromatase inhibitor for postmenopausal women with estrogen receptor-positive breast cancer, but biomarker profiles and treatment tolerability vary among patients. This study described the distributions of *ESR1* rs2234693 and rs9340799 and explored their relationships with letrozole-related biomarkers and self-reported symptoms in an Iraqi cohort.

Materials & Methods: This exploratory cross-sectional pharmacogenetic study included 100 postmenopausal women receiving letrozole 2.5 mg daily for at least three months. *ESR1* variants were genotyped using allele-specific PCR/ARMS-PCR. Estradiol, CA15-3, calcium, osteocalcin, and bone-specific alkaline phosphatase were measured, and treatment-related symptoms were recorded. Genotype associations were examined under the three-genotype, dominant, and recessive models; logistic regression estimates were unadjusted.

Results: For rs2234693, genotype frequencies were TT 29 (29%), TC 28 (28%), and CC 43 (43%); the T and C allele frequencies were 43% and 57%, respectively. The distribution deviated from Hardy–Weinberg equilibrium. All participants carried the rs9340799 AA genotype, precluding association testing. Under the dominant rs2234693 model, TC/CC carriers had higher CA15-3 concentrations than TT carriers (nominal $P = 0.036$; Benjamini–Hochberg false-discovery-rate [FDR] $q = 0.378$). Hair loss was reported in 44.8% of TT carriers and 23.9% of TC/CC carriers (Fisher exact $P = 0.054$). Crude logistic regression yielded an OR of 0.39 (95% CI: 0.16–0.97; $P = 0.042$); after adjustment for age, body mass index, and letrozole duration, the OR was 0.34 (95% CI: 0.13–0.88; $P = 0.026$).

Conclusion: *ESR1* rs2234693 showed substantial variability in this Iraqi cohort, whereas rs9340799 was monomorphic. No genotype–outcome association remained significant after FDR correction. The nominal CA15-3 and hair-loss signals require independent prospective validation before any clinical application.

Keywords: *Breast Neoplasms, Letrozole, Estrogen Receptor alpha, Pharmacogenetics, Polymorphism, Single Nucleotide*

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1. Introduction

Breast cancer is the most frequently diagnosed malignancy in women and remains a major cause of cancer-related morbidity and mortality worldwide. Contemporary global estimates also demonstrate marked differences between regions in incidence, stage at diagnosis, and survival, emphasizing the need for locally generated clinical and molecular evidence (1,2). Hormone receptor-positive disease constitutes the predominant biological subtype of breast cancer. In postmenopausal women, endocrine therapy is a central component of systemic treatment and substantially reduces recurrence risk when integrated appropriately with surgery, radiotherapy, chemotherapy, and other indicated interventions (3-7). Letrozole is a third-generation non-

steroidal aromatase inhibitor that suppresses peripheral estrogen synthesis by blocking the conversion of androgens to estrogens. Its efficacy has been established in randomized trials and is reflected in major clinical guidelines for postmenopausal hormone receptor-positive breast cancer (5-10). Despite its clinical effectiveness, letrozole therapy is accompanied by substantial interindividual variation in biochemical profiles, adverse symptoms, adherence, and long-term outcomes. This heterogeneity is multifactorial and may reflect tumor characteristics, previous treatment, comorbidities, treatment duration, medication adherence, bone-health status, and inherited variation in genes involved in estrogen signaling and endocrine response (11-17). *ESR1* encodes estrogen receptor alpha, a nuclear receptor that regulates estrogen-dependent transcription and interacts with non-genomic signaling pathways. Somatic *ESR1* mutations are established mechanisms of acquired endocrine resistance in advanced breast cancer; however, germline *ESR1* variants may also influence receptor regulation, tissue sensitivity to estrogen deprivation, or susceptibility to treatment-related symptoms (11-17). The intronic variants rs2234693 (PvuII T>C) and rs9340799 (XbaI A>G) have been widely investigated. Although they do not alter the amino-acid sequence, they may serve as markers of regulatory variation or linkage disequilibrium with functional elements affecting *ESR1* expression (18-21). CA15-3 is a MUC1-associated circulating tumor marker that may support monitoring in selected clinical circumstances, but it is not an independent diagnostic test or a definitive pharmacodynamic measure of letrozole response. Aromatase inhibitor-associated symptoms, including musculoskeletal complaints and alopecia, are clinically relevant because they may impair quality of life and adherence; however, they also have multiple genetic and non-genetic determinants (19-27).

Pharmacogenetic evidence concerning endocrine therapy in Iraqi and neighboring Middle Eastern populations remains limited. This exploratory study aimed to address this local knowledge gap by describing the distributions of *ESR1* rs2234693 and rs9340799 and exploring associations between rs2234693 and letrozole-related biomarker and tolerability outcomes among Iraqi postmenopausal women with estrogen receptor-positive breast cancer (28-31).

2. Materials and Methods

2.1 Study Design, Setting, and Participants

This observational, non-interventional, cross-sectional pharmacogenetic study was conducted between 15 October 2025 and 15 January 2026 at Al-Imam Al-Hassan Al-Mujtaba Teaching Hospital, Kerbala Health Directorate, Ministry of Health, Iraq, in collaboration with the College of Pharmacy, University of Kerbala. Letrozole treatment was prescribed independently of the study and was not initiated or allocated by the investigators. A total of 112 Iraqi postmenopausal women aged 45 years or older with histologically confirmed estrogen receptor-positive breast cancer were initially enrolled after meeting the clinical eligibility criteria and providing written informed consent. All had received letrozole 2.5 mg once daily for at least three months. After repeat ARMS-PCR, 12 samples remained uninterpretable and were excluded from the genotype–phenotype analytical cohort; the final cohort therefore comprised 100 women with interpretable genotype and complete biomarker data. Exclusion criteria were premenopausal or perimenopausal status, use of endocrine therapy other than letrozole, chronic hepatic or renal disease, autoimmune or chronic inflammatory disease, poor treatment adherence, unreliable clinical records, metabolic bone disease unrelated to aromatase inhibitor therapy, and concomitant medications considered likely to alter estrogen metabolism or bone turnover.

Eligible participants were enrolled consecutively during the recruitment period. Of the 112 enrolled participants, 100 yielded interpretable genotype calls after repeat testing and constituted the final genotype-phenotype cohort, resulting in an overall genotyping success rate of 89.3% (100/112).

As the sample size of 112 reflected the number of eligible, consenting participants available during the prespecified study period, no formal *a priori* power calculation was performed. An exploratory post-hoc calculation, based on the observed hair-loss proportions in the final cohort (13/29 vs. 17/71), estimated approximately 49% power for a two-sided Fisher's exact test at α=0.05\alpha = 0.05α=0.05. Consequently, the study may have been underpowered to detect small-to-moderate genotype effects; therefore, all genotype-phenotype analyses were considered exploratory. Demographic, clinical, tumor-related, and treatment-related variables were recorded using a structured data-collection form.

2.2 Blood Sampling, Biomarker Measurements, and Symptom Assessment

Venous blood samples were collected for biochemical and molecular analyses. EDTA-anticoagulated whole blood was used for genomic DNA extraction, and serum was separated for laboratory measurements. Estradiol and CA15-3 were quantified by electrochemiluminescence immunoassay on a Roche cobas e platform. Serum calcium was measured by the NM-BAPTA colorimetric method on a Roche cobas c platform. Osteocalcin and bone-specific alkaline phosphatase were measured using double-antibody sandwich ELISA kits according to the manufacturers' instructions. CA15-3 was included as a complementary clinical monitoring biomarker because it may reflect disease activity in selected follow-up settings; it was not considered a direct pharmacodynamic marker of letrozole efficacy. CA15-3 was measured once during ongoing letrozole treatment after at least three months of therapy; pretreatment baseline values, tumor stage, and metastatic status were not available. One result was reported by the laboratory as greater than 300 U/mL and was treated as right-censored in descriptive reporting; a sensitivity analysis excluding that observation was performed for the dominant-model CA15-3 comparison.

Potential letrozole-related symptoms were assessed through patient interviews and the review of available clinical records using a structured checklist. Hot flashes,

joint pain, hair loss, blurry vision, and mood changes were coded as present or absent. An adverse-effect burden score was calculated as the number of recorded symptoms. These outcomes were interpreted as patient-reported tolerability measures rather than adjudicated causal adverse drug reactions, given that a validated symptom questionnaire or standardized dermatological grading instrument was not used (19-27).

2.3 DNA Extraction and *ESR1* Genotyping

Genomic DNA was isolated from whole blood using the Geneaid Blood DNA Extraction Kit. DNA concentration and purity were checked spectrophotometrically before amplification. The primer sequences for *ESR1* rs2234693 and rs9340799 were adopted from Eshaghi et al. (32) and are provided in Supplementary Table S1. Allele-specific amplification was based on the amplification refractory mutation system (ARMS-PCR) principle described by Newton et al. (33). Genotype calling and reporting followed predefined quality-control criteria, with relevant PCR reporting considerations taken into account (34). Each 25-µL reaction contained 10 µL PCR master mix, 4 µL total primers (1 µL each), 8 µL nuclease-free water, and 3 µL DNA template. The cycling conditions consisted of an initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 61°C for 30 seconds, extension at 72°C for 45 seconds, and a final extension at 72°C for 7 minutes. Products were separated on a 2% agarose gel in 1× TBE buffer at 100 V for 45 minutes and visualized using RedSafe nucleic acid stain. For rs2234693, the 368-bp product served as the internal amplification-control band, whereas 293-bp and 124-bp products identified the T- and C-specific alleles, respectively. For every accepted call, both the 368-bp control band and the expected allele-specific band pattern were required. Samples with absent or unclear amplification were repeated. Of the 112 initially enrolled samples, 12 remained uninterpretable after repeat testing and were excluded from the final genotype-phenotype cohort, resulting in an overall genotyping success rate of 89.3% (100/112). The final 100 participants had interpretable results for both loci. While the internal control confirms successful amplification in accepted reactions, it does not replace allele-specific external positive controls or orthogonal validation. Formal blinding of genotype interpretation to clinical data was not documented, and independent validation by sequencing or another orthogonal method was not available; these were addressed as methodological limitations. (Figure1)

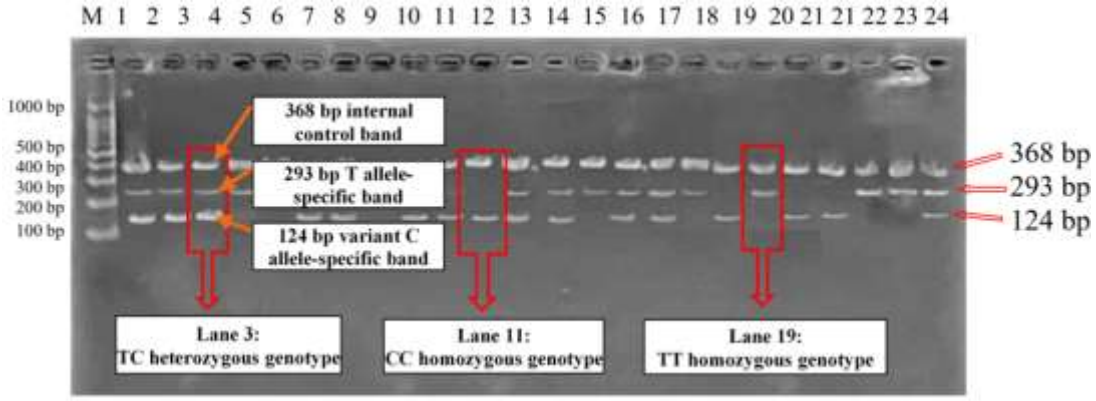


Figure 1. Representative ARMS-PCR agarose gel for *ESR1* rs2234693 genotyping. The 368-bp band is the internal amplification control. The 293-bp and 124-bp bands represent the T- and C-specific alleles, respectively. Lane 3: TC heterozygote (368, 293, and 124 bp); lane 11: CC homozygote (368 and 124 bp); lane 19: TT homozygote (368 and 293 bp); M: 100-bp DNA ladder. The figure demonstrates genotype interpretation in accepted reactions and does not represent all samples. **(Prepared by Authors, 2026)**.

2.4 Statistical Analysis

Data were analyzed using IBM SPSS Statistics version 24.0. Continuous variables were summarized as mean ± standard deviation or median (interquartile range), depending on their distribution. Categorical variables were reported as frequencies and percentages. Genotype and allele frequencies were calculated by direct counting. Hardy-Weinberg equilibrium for rs2234693 was evaluated using an exact test; it did not apply to the monomorphic rs9340799 locus. Given that the cohort consisted of a clinical patient population rather than a random population sample, the equilibrium findings were interpreted cautiously (35-38).

Continuous outcomes were compared across TT, TC, and CC genotypes using the Kruskal–Wallis test. Mann–Whitney U tests were employed for the dominant (TT vs. TC+CC) and recessive (TT+TC vs. CC) models. These genetic models were exploratory rather than pre-specified mechanistic models and were used to describe the observed data comprehensively. Categorical symptom outcomes were analyzed using the Chi-square test or Fisher's exact test, as appropriate. Binary logistic regression was used to estimate crude and adjusted odds ratios (OR) and 95% confidence intervals (CI) for hair loss under the dominant model. The adjusted model included genotype, age, body mass index, and letrozole duration; chemotherapy and radiotherapy were excluded due to sparse unexposed groups, and nutritional status was unavailable. The Benjamini–Hochberg false discovery rate (FDR) correction was applied across 33 genotype–outcome comparisons (five biomarkers and six symptom outcomes, each evaluated under three genetic models). A

two-sided PPP value below 0.05 was considered nominal before multiplicity correction. Reporting was informed by the STROBE and STREGA recommendations (35,36,39).

3. Result

3.1 Participant Characteristics

A total of 112 participants were initially enrolled. Twelve samples remained uninterpretable after repeat genotyping and were excluded from the genotype-phenotype analytical cohort, resulting in a final cohort of 100 postmenopausal women receiving letrozole for estrogen receptor-positive breast cancer (overall genotyping success rate: 89.3%). Complete data were available for all variables included in the genotype-biomarker and genotype-symptom analyses within this cohort. Mean age was 57.15 ± 8.68 years and mean body mass index was 27.30 ± 5.64 kg/m2. Most participants were married, were non-smokers, had undergone mastectomy, and had previously received chemotherapy and radiotherapy. Baseline characteristics are summarized in Table 1.

Table 1. Demographic, clinical, tumor, and treatment characteristics of the study cohort (N=100).

Variable	Value
Age, years	57.15 ± 8.68
BMI, kg/m2	27.30 ± 5.64
Number of children	Median 4 (IQR 2-6)
Marital status: married / widowed / single	90 (90%) / 4 (4%) / 6 (6%)
Smoking: smoker / non-smoker	6 (6%) / 94 (94%)
Disease duration, years	Median 4 (IQR 2-6); mean 4.27 ± 2.50
Letrozole duration, months	Median 7 (IQR 5-9); mean 8.00 ± 3.96
Family history of breast cancer	63 (63%)
Diabetes mellitus	39 (39%)
Hypertension	55 (55%)
HER2-positive / HER2-negative	29 (29%) / 71 (71%)
Tumor laterality: bilateral / left / right	3 (3%) / 60 (60%) / 37 (37%)
Mastectomy / breast-conserving surgery	96 (96%) / 4 (4%)
Previous chemotherapy	96 (96%)
Previous radiotherapy	85 (85%)

3.2 *ESR1* Genotype and Allele Distributions

For rs2234693, CC was the most frequent genotype (43%), followed by TT (29%) and TC (28%). The C allele frequency was 57%, and the T allele frequency was 43%. The observed distribution deviated from Hardy–Weinberg equilibrium (exact P<0.001P < 0.001P<0.001), primarily due to a lower-than-expected heterozygote count. In this disease-based, consecutively recruited cohort, factors such as population structure, selection, non-random sampling, disease association, and genotyping error may explain this deviation; thus, the result must not be interpreted as evidence of a biological association. For rs9340799 (A>G), A was treated as the reference allele and G as the alternate allele. All participants in the final cohort carried the AA genotype; consequently, this locus was presented descriptively and excluded from genotype-phenotype analyses (Table 2).

Table 2. *ESR1* rs2234693 and rs9340799 genotype and allele distributions.

Variant	Genotype/allele	Observed n	Observed %	Expected n / interpretation
rs2234693	TT	29	29.0	18.49
rs2234693	TC	28	28.0	49.02
rs2234693	CC	43	43.0	32.49
rs2234693	T allele	86	43.0	-
rs2234693	C allele	114	57.0	HWE exact P<0.001
rs9340799	AA	100	100.0	Monomorphic
rs9340799	AG	0	0.0	Association testing not applicable
rs9340799	GG	0	0.0	-
rs9340799	A allele	200	100.0	-
rs9340799	G allele	0	0.0	-

3.3 Biomarker Outcomes According to *ESR1* rs2234693

No biomarker differed significantly across the three individual genotypes. CA15-3 medians were 23.93 (IQR 16.20-29.74) U/mL in TT, 29.34 (20.04-44.11) U/mL in TC, and 25.78 (23.52-32.46) U/mL in CC carriers (P=0.081). Under the dominant model, TC+CC carriers had higher CA15-3 values than TT carriers (nominal P=0.036; FDR q=0.378). This result was similar after exclusion of the single right-censored CA15-3 value (P=0.045). CA15-3 was not correlated with letrozole duration (Spearman rho=-0.081, P=0.421). Estradiol, calcium, osteocalcin, and bone-specific alkaline phosphatase showed no nominally significant dominant-model differences, and no biomarker association remained significant after FDR correction (Table 3).

Table 3. Biomarker outcomes according to *ESR1* rs2234693 genetic models.

Outcome	TT median (IQR)	TC+CC median (IQR)	Three-genotype P	Dominant P	Recessive P	Interpretation after FDR correction
CA15-3, U/mL	23.93 (16.20-29.74)	27.02 (22.15-36.44)	0.083	0.036	0.621	No FDR-significant association
Estradiol, pg/mL	21.04 (15.69-36.46)	19.94 (15.10-31.13)	0.202	0.319	0.074	No FDR-significant association
Calcium, mg/dL	9.11 (8.95-9.34)	9.30 (8.90-9.70)	0.370	0.310	0.170	No FDR-significant association
Osteocalcin, ng/mL	21.00 (18.95-31.05)	23.50 (17.80-32.10)	0.294	0.974	0.189	No FDR-significant association
Bone-specific ALP, U/L	18.42 (15.54-20.59)	19.20 (17.13-21.31)	0.420	0.277	0.225	No FDR-significant association

Note: P values were obtained using the Kruskal-Wallis test for the three-genotype model, and Mann-Whitney U tests for dominant and recessive models. Benjamini-Hochberg FDR correction was applied across all 33 genotype-outcome comparisons; the complete nominal and FDR-adjusted results are provided in Supplementary Table S2. No association remained significant after correction.

3.4 Letrozole-Related Tolerability Outcomes

Hair loss was reported by 30 participants, and blurry vision by 21. Under the dominant model, none of the individual symptoms reached statistical significance in the group comparisons. Hair loss was less frequent among TC+CC carriers than TT carriers (23.9% vs. 44.8%; Fisher's exact P=0.054P = 0.054P=0.054). In crude logistic regression, TC+CC carriers had lower odds of reported hair loss than TT carriers (OR = 0.39; 95% CI: 0.16–0.97; P=0.042P = 0.042P=0.042). In an exploratory adjusted model including age, body mass index, and letrozole duration, the association remained similar (adjusted OR = 0.34; 95% CI: 0.13–0.88; P=0.026P = 0.026P=0.026). This result was not interpreted as confirmatory, as the symptom was patient-reported, only 30 events occurred, and no genotype–outcome associations survived FDR correction. The recessive model was not significant, and the adverse-effect burden score did not differ between groups (Table 4).

Table 4. Dominant-model comparison of reported symptoms and logistic regression for hair loss.

Reported outcome	TT (n=29)	TC+CC (n=71)	Group-comparison P	Crude OR (95% CI); P / Adjusted OR (95% CI); P
Hot flashes, n (%)	17 (58.6%)	51 (71.8%)	0.240	-
Joint pain, n (%)	25 (86.2%)	62 (87.3%)	1.000	-
Hair loss, n (%)	13 (44.8%)	17 (23.9%)	0.054	Crude: 0.39 (0.16-0.97); 0.042 Adjusted*: 0.34 (0.13-0.88); 0.026
Blurry vision, n (%)	7 (24.1%)	14 (19.7%)	0.601	-
Mood changes, n (%)	6 (20.7%)	17 (23.9%)	0.799	-
Adverse-effect burden, median (IQR)	2 (2-3)	2 (2-3)	0.975	-
Hair loss: recessive model	-	CC vs TT+TC	-	0.45 (0.18-1.13); 0.089

Note: Categorical outcomes were compared with chi-square or Fisher's exact tests; the burden score was compared with the Mann-Whitney U test. Logistic regression estimates were reported as crude and, for hair loss under the dominant model, adjusted for age, body mass index, and letrozole duration. All genotype-outcome findings were interpreted after FDR correction.

***Adjusted for age, body mass index, and letrozole duration. The adjusted model was exploratory; chemotherapy and radiotherapy were not included because of sparse unexposed groups.**

4. Discussion

This exploratory study provides pharmacogenetic data on two *ESR1* variants among Iraqi postmenopausal women receiving letrozole for estrogen receptor-positive breast cancer. The principal findings were substantial variability in rs2234693, a monomorphic distribution of rs9340799, and nominal dominant-model signals for CA15-3 and reported hair loss. However, neither signal remained significant after Benjamini–Hochberg FDR correction across the tested genotype–outcome comparisons; therefore, no finding should be viewed as confirmatory or clinically actionable.

The rs2234693 genotype distribution demonstrated that this locus was informative within the study cohort; however, the marked deficit of heterozygotes resulted in a deviation from Hardy–Weinberg equilibrium. In a disease-based sample, such deviation can arise from

population structure, selection, non-random sampling, disease association, or genotyping error; it therefore cannot be assumed to reflect a biological association. While the use of internal-control bands, repetition of unclear amplifications, and predefined band interpretation reduced the probability of misclassification, it did not eliminate it. Independent confirmation via Sanger sequencing, real-time allelic discrimination, or another orthogonal method is essential before these frequency estimates can be generalized **(28,33-38)**.

The finding that all 100 participants in the final cohort carried the rs9340799 AA genotype warrants particular restraint. The A allele is the reference allele, and G is the alternate allele. In gnomAD v4 genome data, the G allele is observed at 40.8% (120/294 alleles) in the Middle Eastern ancestry group, whereas it was not observed in the present cohort. This comparison is contextual only: the gnomAD Middle Eastern group is relatively small, is not Iraqi-specific, and does not establish an expected frequency for Iraqi patients. Nevertheless, the discrepancy reinforces the need for independent replication and orthogonal validation. A genuinely low local G-allele frequency, cohort-specific selection, sampling variability, or technical limitations may each contribute to an apparently monomorphic result. Because the locus lacked genotype variability, no valid genotype–phenotype inference could be made. The present observation should not be generalized beyond this cohort **(28,41-43)**.

The biological rationale for examining *ESR1* remains plausible. Estrogen receptor alpha coordinates ligand-dependent transcription and interacts with growth-factor and non-genomic signaling pathways that shape endocrine sensitivity. Unlike acquired somatic *ESR1* mutations in advanced breast cancer, rs2234693 and rs9340799 are germline intronic variants and do not directly alter the receptor amino acid sequence. Their potential effects are more likely to involve regulatory context or linkage disequilibrium with functional variants. Consequently, any association with letrozole outcomes is expected to be modest and population-dependent rather than determinative **(11-18)**.

The higher CA15-3 concentration observed among TC+CC carriers under the dominant model should be considered a hypothesis-generating signal only. Because CA15-3 levels were obtained during treatment rather than at baseline, and the study lacked data on tumor stage, metastatic status, serial measurements, radiological response assessment, and time-to-event outcomes, the elevated concentration may reflect tumor burden, treatment history, assay variability, or other clinical factors rather than a letrozole-related pharmacodynamic effect. The dominant-model nominal P value was 0.036 (FDR q=0.378), and the sensitivity analysis excluding the single value reported as >300 U/mL was similar (P=0.045). These data do not establish clinical meaning or support therapeutic decisions **(22,23,26,27)**.

The direction of the hair-loss association may be clinically interesting, as endocrine therapy-induced alopecia can affect body image, quality of life, and adherence. Nevertheless, the group comparison did not reach conventional statistical significance (Fisher's exact P=0.054P = 0.054P=0.054). The adjusted model—incorporating age, body mass index, and letrozole duration—yielded an adjusted OR of 0.34 (95% CI: 0.13–0.88; P=0.026P = 0.026P=0.026), but this must be interpreted cautiously: only 30 events were available, the confidence interval was wide, the symptom was patient-reported rather than graded using a validated instrument, and the broader genotype–outcome testing framework did not achieve FDR significance. Potential confounders, such as nutritional status, pre-treatment hair status, and tumor stage, were unavailable, while chemotherapy and radiotherapy exhibited limited variability. The result is, therefore, insufficient to suggest a protective genetic effect.

No significant genotype differences were found for estradiol or the bone-related biomarkers. A single estradiol measurement may not fully reflect cumulative estrogen suppression, medication adherence, assay performance at very low postmenopausal concentrations, or tissue-level receptor signaling. Likewise, serum calcium, osteocalcin, and bone-specific alkaline phosphatase provide supportive biochemical information but do not replace bone mineral density assessment or structured fracture-risk evaluation. Contemporary recommendations emphasize clinical risk assessment and appropriate bone-targeted management rather than reliance on isolated biochemical measurements **(6-10,24-27)**.

The study has several strengths. It addresses an understudied population, reports genotype and allele frequencies, separates biomarker and tolerability domains, provides reproducible ARMS-PCR details, and interprets findings as exploratory rather than clinically predictive. The collaboration between pharmacology, molecular laboratory, and oncology services also supported the accurate characterization of the treatment context.

The limitations are substantial. The cross-sectional design precludes causal inference and does not capture longitudinal biomarker changes, objective treatment response, recurrence, progression-free survival, or overall survival **(44,45)**. Twelve of the 112 initially enrolled participants were excluded because repeat genotyping remained uninterpretable, resulting in an overall call rate of 89.3%; this may have introduced selection bias and limits generalizability. The sample size was constrained by feasibility; the exploratory post-hoc power for the observed hair-loss difference was approximately 49%, meaning the absence of a statistically significant association should not be interpreted as evidence of absence. No associations remained significant after FDR correction. Furthermore, tumor stage, metastatic status, pretreatment CA15-3 values, nutritional status, baseline hair status, and validated symptom instruments were unavailable. Logistic regression was necessarily parsimonious; *rs9340799* could not be analyzed because it was monomorphic, and genotypes were not independently verified. Larger multicenter studies should incorporate orthogonal genotype validation, prespecified outcomes, serial biomarkers, imaging-based response assessment, standardized adverse-effect instruments, and

adequately powered multivariable analyses **(19-21,36-43)**.

5. Conclusion

ESR1 rs2234693 showed substantial genetic variability among Iraqi postmenopausal women with estrogen receptor-positive breast cancer receiving letrozole, whereas rs9340799 was monomorphic in this cohort. Nominal dominant-model signals were observed for CA15-3 and reported hair loss, but no genotype–outcome associations remained significant after FDR correction. In view of the Hardy–Weinberg disequilibrium, lack of orthogonal genotype validation, cross-sectional sampling, limited power, unavailable tumor-stage data, and reliance on patient-reported symptoms, these findings do not support clinical decision-making based on either variant. Independent prospective validation is required.

6. Declarations

6.1 Acknowledgments

The authors thank the participating patients and the staff of Al-Imam Al-Hassan Al-Mujtaba Teaching Hospital for their cooperation and support during recruitment and sample collection.

6.2 Ethical Considerations

Ethical approval was granted by the Research Management Office, Training and Human Development Centre, Kerbala Health Directorate, Ministry of Health, Iraq (official clearance letter 6/3176, dated 12 October 2025). Trial registration was not applicable.

6.3 Authors' Contributions

Conceptualization, S.H.S. and Z.A.A.; methodology, S.H.S., Z.A.A., and S.A.J.; investigation and data collection, S.H.S. and A.A.A.M.; laboratory coordination, S.H.S.; formal analysis, S.H.S.; clinical assessment and resources, A.A.A.M.; interpretation, S.H.S., Z.A.A., S.A.J., and A.A.A.M.; writing-original draft preparation, S.H.S.; writing-review and editing, Z.A.A., S.A.J., and A.A.A.M.; supervision, Z.A.A. All authors read and approved the final manuscript.

6.4 Conflict of Interest

The authors declare no conflict of interest.

6.5 Fund or Financial Support

This research received no external funding.

6.6 Using Artificial Intelligence Tools (AI Tools)

During the revision of this manuscript, the authors used Claude (Anthropic) to assist with checking the reference list, citation numbering, and the consistency of author names in citations. The authors reviewed and verified all outputs and take full responsibility for the content of this article.

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