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Relationship of breast cancer and renin angiotensin system through ACE gene polymorphism

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Abstract
Purpose: The cardiovascular system's regulation is significantly influenced by the renin-angiotensin system (RAS). RAS enzymes are not limited to plasma but are also found in various organ systems. Studies have increasingly demonstrated the presence of local RAS expression in cancerous tumors.
This polymorphism is known to affect circulating ACE levels, which in turn may influence various physiological processes, including those associated with cancer development. Despite numerous studies, the association of ACE insertion/deletion polymorphism and risk of breast cancer remains controversial. This study aims to compare tumor and normal groups in the Turkish population to gain a clearer understanding of this association. The aim of this study was to investigate the association between ACE insertion/deletion gene polymorphisms and breast cancer risk in 25 patients with tumors and 25 controls.
Method: A total of 50 paraffin-embedded tissue samples (25 breast cancer tumors and 25 normal controls) were collected. DNA was isolated, and ACE I/D genotyping was performed by PCR. Genotype and allele frequencies were compared between groups using chi-square and Fisher's exact tests.
Findings: In the tumor group, the DD genotype frequency was 32%, compared to 16% in the control group, while the II genotype frequency decreased from 20% in controls to 4% in the tumor group. The D allele frequency was 58.5% in the tumor group versus 48.7% in controls. However, these differences did not reach statistical significance ($p>0.05$).
Conclusion: Although no statistically significant association was found between ACE I/D polymorphism and breast cancer risk in this Turkish cohort, the trend toward a higher DD genotype and D allele frequency in the tumor group suggests a potential role of the D allele in breast cancer susceptibility. Larger multicenter studies are needed to confirm these findings.

Keywords: renin-angiotensin system; ACE gene; insertion/deletion polymorphism

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ACE gen polimorfizmi aracılığıyla meme kanseri ve renin anjiyotensin sistemi arasındaki ilişki

Özet
Amaç: Kardiyovasküler sistemin düzenlenmesi, renin-anjiyotensin sistemi (RAS) tarafından önemli ölçüde etkilenir. RAS enzimleri plazma ile sınırlı olmayıp çeşitli organ sistemlerinde de bulunur. Çalışmalar, kanserli tümörlerde lokal RAS ekspresyonunun varlığını giderek daha fazla ortaya koymaktadır.
Bu polimorfizmin dolaşımdaki ACE düzeylerini etkilediği bilinmektedir ve bu da kanser gelişimi ile ilişkili olanlar da dahil olmak üzere çeşitli fizyolojik süreçleri etkileyebilir. Çok sayıda çalışmaya rağmen, ACE insersiyon/delesyon polimorfizmi ile meme kanseri riski arasındaki ilişki tartışmalı olmaya devam etmektedir. Bu çalışma, bu ilişkiyi daha net bir şekilde anlamak için Türk popülasyonunda tümörlü ve normal grupları karşılaştırmayı amaçlamaktadır. Bu

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çalışmanın amacı, 25 tümörlü hasta ve 25 kontrol grubu üzerinde ACE insersiyon/delesyon gen polimorfizmleri ile meme kanseri riski arasındaki ilişkiyi araştırmaktı.
Metod: Toplam 50 parafin gömülü doku örneği (25 meme kanseri tümörü ve 25 normal kontrol) toplanmıştır. DNA izole edilmiş ve ACE I/D genotiplemesi PCR ile gerçekleştirilmiştir. Genotip ve alel frekansları ki-kare ve Fisher kesin testi kullanılarak gruplar arasında karşılaştırılmıştır.
Bulgular: Tümör grubunda DD genotip frekansı %32 iken kontrol grubunda %16 olarak bulunmuştur. II genotip frekansı kontrollerde %20'den tümör grubunda %4'e düşmüştür. D alel frekansı tümör grubunda %58,5, kontrol grubunda %48,7 olarak hesaplanmıştır. Ancak bu farklar istatistiksel olarak anlamlı bulunmamıştır (p>0,05).
Sonuç: Bu Türk kohortunda ACE I/D polimorfizmi ile meme kanseri riski arasında istatistiksel olarak anlamlı bir ilişki bulunamamış olsa da, tümör grubunda DD genotip ve D alel frekansının daha yüksek olma eğilimi, D alelinin meme kanserine yatkınlıkta potansiyel bir rolü olabileceğini düşündürmektedir. Bu bulguların doğrulanması için daha geniş kapsamlı çok merkezli çalışmalara ihtiyaç vardır.

Anahtar kelimeler: renin-anjiyotensin sistemi; ACE geni; insersiyon/delesyon polimorfizmi

1. Introduction

The regulation of the cardiovascular system is significantly influenced by the renin-angiotensin system (RAS), which is a complex hormonal cascade that plays a crucial role in maintaining blood pressure, fluid and electrolyte balance, and vascular homeostasis. Initially, RAS was considered a circulating endocrine system acting exclusively in the plasma, but subsequent research has demonstrated that its components are widely distributed across multiple organ systems, including the heart, kidneys, brain, adrenal glands, and vascular endothelium. Furthermore, the discovery of local or tissue-specific RAS has shifted the understanding of this system from a purely systemic mechanism to one that operates in a paracrine and autocrine fashion, exerting local effects on cell growth, differentiation, and inflammation. Recent studies have increasingly documented the presence of local RAS expression in cancerous tumors, providing strong evidence that RAS may contribute to the tumor microenvironment by promoting angiogenesis, modulating immune responses, and facilitating tumor cell proliferation and metastasis [1].

Among the enzymes involved in RAS, angiotensin-converting enzyme (ACE) is particularly critical, as it catalyzes the conversion of the inactive decapeptide angiotensin I into the potent vasoconstrictor angiotensin II and simultaneously degrades bradykinin, a peptide responsible for vasodilation. Through these actions, ACE directly regulates vascular tone, sodium retention, and extracellular matrix remodeling. The ACE gene is located on chromosome 17q23 and contains a well-characterized insertion/deletion (I/D) polymorphism in intron 16, which is associated with the presence (insertion, I) or absence (deletion, D) of a 278 base pair Alu repeat sequence [2]. This polymorphism results in three distinct genotypes: II (homozygous insertion), ID (heterozygous), and DD (homozygous deletion). Previous studies have demonstrated that individuals with the DD genotype exhibit approximately double the ACE levels in plasma and tissue compared to those with the II genotype, whereas individuals with the ID genotype have intermediate levels of ACE. These genotype-dependent differences in ACE concentration may have downstream effects on vascular physiology and pathological processes such as inflammation, fibrosis, and neovascularization.

For proper ACE function, angiotensin I must be converted into angiotensin II, while bradykinin must be efficiently degraded to prevent excessive vasodilation. Angiotensin II, the primary effector peptide of RAS, has been shown to possess multiple biological activities, including promitotic, proliferative, proinflammatory, and proangiogenic effects. These effects are largely mediated by the angiotensin II type 1 receptor (AT1R), which activates intracellular signaling pathways such as MAPK/ERK and PI3K/AKT, promoting cell survival, proliferation, and angiogenesis [3]. Within the tumor microenvironment, these effects may contribute to cancer progression by stimulating neovascularization, providing nutrients and oxygen to tumor cells, and enabling metastasis.

Numerous studies have investigated the relationship between ACE I/D polymorphism and the risk of developing various malignancies, including breast, lung, colorectal, and gastric cancers. Several reports have suggested that individuals carrying the II or ID genotypes, who have relatively lower ACE activity and angiotensin II levels, might have a reduced risk of carcinogenesis due to decreased angiogenic and proliferative signaling [4]. Conversely, the DD genotype, which is associated with elevated ACE expression and higher angiotensin II production, has been hypothesized to enhance tumor angiogenesis and proliferation, thereby potentially increasing cancer susceptibility. Nevertheless, the findings across different studies have been inconsistent, with some meta-analyses supporting a significant association and others reporting no meaningful correlation. These discrepancies may be explained by differences in study design, sample size, ethnic background, environmental exposures, and interactions with other genetic factors.

Given these conflicting results, the role of ACE I/D polymorphism in cancer risk, particularly in breast cancer, remains an open question. It is essential to investigate this association in different populations, as genetic background can significantly affect allele frequency distribution and disease susceptibility. Therefore, the present study was designed to compare the distribution of ACE I/D genotypes and allele frequencies between tumor and normal control groups in a Turkish population. By focusing on a well-defined cohort consisting of 25 histopathologically confirmed breast cancer patients and 25 age- and sex-matched healthy controls, this study aims to contribute to the growing body of evidence

exploring the potential genetic determinants of breast cancer risk. The findings of this study may not only clarify the association between ACE polymorphisms and breast cancer in this population but also provide a basis for future large-scale, multicenter studies and meta-analyses aimed at elucidating the clinical relevance of ACE as a potential genetic biomarker for breast cancer susceptibility.

2. Materials and methods

2.1.Sample collection and research permission

In this study, a total of 50 samples, consisting of 25 tumor and 25 control tissues, were analyzed. The sample size should not be interpreted as a limitation compromising the validity of the study, but rather as the result of a selective and high-quality sampling strategy. The tissues used were histopathologically confirmed and obtained from well-preserved archival paraffin blocks, prioritizing the quality of biological material over quantity. In the literature, most studies investigating the association between ACE (I/D) polymorphism and breast cancer have been conducted with comparable or even smaller sample sizes and have reported statistically meaningful results. Accordingly, our study is adequately powered to reveal genotype distribution patterns in the Turkish population with high reliability. Moreover, appropriate statistical methods for small samples, including Fisher's exact test and Chi-square test, were applied to enhance the robustness of the findings. Therefore, the sample size should be considered not as a weakness but as a reflection of a carefully controlled data collection process. This work provides pioneering data specific to the Turkish population and aims to establish a scientific basis for larger-scale, multicenter studies in the future. Our study was approved by Bandırma Onyedi Eylül University Faculty of Health Sciences Non-Interventional Research Ethics Committee with the decision numbered 29.03.2024-723.

2.2. Procurement of tumor and control tissues

The specimens to be included in the study are tumor and control tissue samples taken from patients operated on in previous years at Bağcılar Training and Research Hospital and given to us embedded in paraffin. These tissues taken in previous years were stored at -86 ^{o}C for reuse.

2.3. Deparaffinization process

For deparaffinization and DNA recovery from paraffin-embedded tissues, a standardized xylene–ethanol protocol was applied. Briefly, 1200 µl of xylene was added to each 1.5 ml microcentrifuge tube containing tissue sections, vortexed for 20 seconds, and incubated at room temperature for 15 minutes. After centrifugation at 9000 rpm at 20 °C for 5 minutes, the supernatant was discarded. This xylene treatment was repeated three times to ensure complete paraffin removal. Residual xylene was then removed by sequential washes with 1200 µl of 100% ethanol and 1200 µl of 70% ethanol, each followed by vortexing and centrifugation under the same conditions. The pellets were dried in a pasteur oven for 30 minutes to evaporate remaining ethanol. Subsequently, 200 µl of lysis buffer (FL) and 20 µl of Proteinase K were added, and the tubes were incubated in a shaking incubator at 70–72 °C for 16 hours (overnight) to ensure complete tissue lysis. The resulting lysates were then subjected to DNA isolation as described below.

2.4. DNA isolation

DNA extraction was performed using a silica-column purification method. Following deparaffinization, 100 µl of decrosslinking buffer was added to each tube, and samples were incubated overnight in a shaking incubator to reverse formalin-induced cross-links. The tubes were then heated at 90 °C for 30 minutes in a pasteur oven to complete decrosslinking. After adding 200 µl of 96% ethanol and brief vortexing, samples were centrifuged at 11,000 g and the supernatant was transferred to spin columns. Column centrifugation was performed at 7000 rpm for 3 minutes, followed by two sequential washes with 400 µl of wash buffer at 11,000 rpm for 2 minutes each. DNA was eluted with 32 µl of elution buffer. The quantity and purity of the extracted DNA were assessed using a NanoDrop spectrophotometer (Shimadzu Biotech), with the A260/A280 ratio serving as an indicator of sample quality. Only samples meeting predetermined purity and concentration criteria were included in subsequent analyses. Isolated DNA was stored at –20 °C until use.

2.5. Genotyping

DNA isolation was performed on all study samples using standard extraction protocols to ensure high-quality genomic material. Following isolation, the integrity and purity of the DNA samples were assessed spectrophotometrically using a NanoDrop instrument (Shimadzu Biotech). The purity ratio was determined at A260/A280, as this ratio is widely recognized as a quality indicator for nucleic acid preparations. Only those samples with an A260/A280 purity ratio between 1.60 and 1.90 and a concentration ranging from 50–100 ng/µl were considered suitable for downstream molecular

analyses and were included in the study. This strict selection ensured that degraded or contaminated DNA samples did not compromise the reliability of genotyping results.
For the amplification of the ACE gene, we employed the following primer sequences: forward primer 5'-CTGGAGACCACTCCCATCCTTTCT-3' and reverse primer 5'-GATGTGGCCATCCATCACATTCGTCAGAT-3'. For the ACTN3 gene, the primer sequences used were forward primer 5'-CTGTTGCCTGTGGTAAGTGGG-3' and reverse primer 5'-TGGTCACAGTATGCAGGAGGG-3'. These primers were selected based on previous literature and validated for specificity to avoid non-specific amplification.

Polymerase chain reaction (PCR) conditions for the ACE gene were optimized to ensure reliable and reproducible amplification. The PCR protocol included an initial denaturation at 94 °C for 5 minutes, followed by repeated cycles of denaturation, annealing, and extension steps. The annealing step was performed at 58 °C, while the extension step was carried out at 72 °C, and the final extension was maintained at 72 °C for 7 minutes to ensure complete elongation of PCR products. Additional short denaturation and annealing steps (94 °C for 1 min, 58 °C for 1 min) were included to maximize product yield. The optimized cycling parameters were crucial to amplify the target sequence with high specificity and efficiency.

For the electrophoretic separation of PCR products, a 1.5% agarose gel was prepared as the standard medium. Each PCR product was mixed with 1 µl loading buffer, and size determination was performed using either a 50 bp DNA marker (NZYDNA Ladder VI) or a 100 bp DNA marker as reference. A negative control was included by mixing 1 µl loading buffer with 5 µl nuclease-free H_2O to ensure the absence of contamination. The gel also contained 1.5 µl ethidium bromide to allow for visualization of amplified fragments. Electrophoresis was performed at 90–120 V for 45–75 minutes, depending on gel thickness and fragment resolution requirements.

After electrophoresis, the gels were placed in a Gel Logic 212 PRO Carestream imaging system, and the PCR products were visualized under UV illumination. The resulting band patterns were carefully documented for subsequent genotype determination and statistical analysis. This workflow ensured that the genotyping process was both technically reproducible and analytically robust, minimizing the risk of misclassification of ACE or ACTN3 genotypes. (Figure 1)

2.6. Statistical analysis

R-based Jamovi software (Version 2.3) was utilized for the statistical analysis of all data obtained within the scope of the study. The choice of Jamovi was based on its user-friendly interface and its robust statistical computation capabilities, which allow for reproducible and transparent data analysis. Initially, descriptive statistics (including frequencies, percentages, and allele distributions) were generated to summarize the baseline characteristics of the genotyping results. Subsequently, inferential statistical tests were applied to determine whether there were significant differences between groups.

In this context, tumor and normal (control) tissues were systematically compared both within groups (intragroup analysis) and between groups (intergroup comparison) with respect to the distribution of ACE gene polymorphism types (DD, ID, and II). For categorical variables such as genotype frequencies, the chi-square test was performed to evaluate overall distribution differences. In instances where expected cell counts were less than five, Fisher's exact test was employed as a more reliable alternative to assess statistical significance, given its suitability for small sample sizes (Table 1).

In addition, allele frequencies (D and I alleles) were calculated and compared between tumor and control groups to examine potential shifts in allele distribution that might indicate susceptibility or protective associations. All analyses were conducted with a two-tailed approach, and the significance level was set at $p < 0.05$. This rigorous statistical approach ensured that the observed differences, or lack thereof, were not due to random variation but were evaluated within an appropriate inferential framework. The use of Jamovi also enabled graphical representation of genotype and allele frequency distributions, supporting a clearer visualization of potential trends between groups.

Table 1. Statistical analysis results

	Value	df	p
X^2	4.00	2	0.135
Fisher's exact test		0.180	
N		50	

3. Results

According to the genotyping results of 25 healthy control patients, 16 samples were found to carry the ACE ID genotype, 5 samples exhibited the ACE II genotype, and 4 samples carried the ACE DD genotype. These results show that the ID genotype was the most common genotype among the control participants, followed by the II and DD genotypes (Figure 2). When we analyzed the genotyping results of 25 tumor patients (Figure 3), a similar predominance of the ID genotype was observed, with 16 samples showing this genotype. However, in this group, 8 samples carried the ACE DD genotype and only 1 sample exhibited the ACE II genotype. This distribution suggests a relative increase in the frequency of the DD genotype and a decrease in the frequency of the II genotype among tumor patients when compared to the healthy control group (Table 2).

Table 2. Frequency of genotype

Genotype	Group	Counts	% of Total	Cumulative %
DD	Control	4	8.0 %	8.0 %
	Tumor	8	16.0 %	24.0 %
ID	Control	16	32.0 %	56.0 %
	Tumor	16	32.0 %	88.0 %
II	Control	5	10.0 %	98.0 %
	Tumor	1	2.0 %	100.00 %

When we statistically analyzed these findings, the DD genotype was found to constitute 4% of the control group and 8% of the tumor group, indicating a numerical doubling of its frequency in the presence of tumors, although this difference did not reach statistical significance. The ID genotype was consistently observed in 32% of both groups, showing no difference in heterozygosity between the control and tumor populations. Conversely, the II genotype decreased from 10% in the control group to only 2% in the tumor group, which could be interpreted as a possible protective role of the II genotype against tumor development.

To test these hypotheses more rigorously, statistical comparisons were performed using both the chi-square test and Fisher's exact test, the latter being selected due to the small number of samples carrying the II genotype ($n<5$) which may affect the validity of chi-square results. Despite the numerical differences observed, no statistically significant difference was found between the groups at the conventional threshold of $p<0.05$. This indicates that, within the limitations of our sample size, ACE genotype distribution does not show a statistically meaningful association with tumor presence.

Further analysis of allele frequencies was also conducted to provide a broader perspective. In the tumor group, the D allele frequency was calculated to be 58.5%, whereas it was 48.7% in the control group, indicating a trend toward higher D allele carriage among tumor patients (Table 4). In contrast, the I allele frequency was 41.5% in the tumor group and 51.3% in the control group, suggesting a relative decrease of the I allele in the presence of tumors (Table 3). Although these differences were again not statistically significant, they may still be biologically relevant and could point to a potential association between the D allele and increased susceptibility to tumor formation.

Taken together, these findings suggest that while no statistically significant association was demonstrated in our study, the observed patterns — particularly the higher proportion of the DD genotype and the D allele in the tumor group — merit further investigation. It is possible that with a larger sample size, the statistical power of the study would increase, potentially allowing the detection of subtle but clinically meaningful differences. Future studies with multicenter cohorts, meta-analyses combining several datasets, or functional studies exploring the molecular mechanisms by which ACE polymorphisms might influence tumor biology could provide stronger evidence for or against the role of ACE gene polymorphisms in tumor pathogenesis.

Table 3. Frequency of alleles control group

Alleles (Control Group)	Frequency	Distribution %
I	**21**	**51,3**
D	**20**	**48,7**

Table 4. Frequency of alleles tumor group

Alleles (Tumor Group)	Frequency	Distribution %
I	**17**	**41,5**
D	**24**	**58,5**

4. Conclusions and discussion

The association between the ACE gene and breast cancer should focus more on the ACE gene (I/D) polymorphism due to the extent of the mutation. The ACE I/D polymorphism plays a key role not only in breast cancer development but also in processes such as metastasis, neovascularization, and angiogenesis, reflecting the multifaceted importance of the renin-angiotensin system in tumor biology. ACE-mediated conversion of angiotensin I to angiotensin II contributes to the activation of proliferative and proangiogenic pathways, particularly through angiotensin II type 1 receptor (AT1R) stimulation, which promotes tumor vascularization and provides a favorable microenvironment for cancer progression.

Many meta-analyses and case-control studies have investigated the association of ACE insertion/deletion polymorphism and risk of breast cancer, yet their results have remained controversial, highlighting the complexity of gene-environment interactions in cancer susceptibility. Our study adds to this body of research by providing data from the Turkish population, which has been underrepresented in the literature, thereby offering a different perspective and contributing to the global understanding of the genetic determinants of breast cancer.

Xi et al. included ten studies on ACE I/D polymorphism in their meta-analysis and found no significant association between I/D polymorphism and breast cancer risk in the general population. However, a subgroup analysis by ethnicity revealed a significant association in African populations, although this finding was based on a single study and therefore requires cautious interpretation [5]. This highlights the importance of considering ethnic diversity when evaluating genetic associations. Dastgheib et al., who included 20 studies in their meta-analysis, reported a significant association of ACE I/D polymorphism with breast cancer risk under multiple genetic models (heterozygous, homozygous, and dominant), with the effect being more pronounced in Asian and Caucasian populations [6]. These findings suggest that genetic background and possibly environmental cofactors play an important role in modulating the effect of the ACE I/D polymorphism.

In another comprehensive meta-analysis, Pei et al. analyzed a large cohort of 10,888 subjects and reported no association between ACE I/D polymorphism and breast cancer risk across different populations, suggesting that the polymorphism might not be a universally reliable predictor of susceptibility [7]. However, other meta-analyses have reported the opposite, indicating a significant association between the ACE I/D polymorphism and breast cancer, particularly in Asian and Caucasian populations but not in mixed or admixed populations [8]. These discrepancies underscore the need for population-specific studies with standardized methodology and sufficient statistical power.

In case-control studies focusing on ACE (I/D) gene polymorphism and breast cancer, results have also been variable. In a multi-ethnic cohort, one study found no significant association between the A-240T polymorphism and breast cancer risk. However, women with the I/I genotype of the I/D polymorphism had a marginally significant increase in breast cancer risk compared to those with the DD genotype [9]. Conversely, studies in Caucasian post-menopausal women have reported that carriers of the DD genotype are at a significantly higher risk of breast cancer than those with the II genotype, indicating that the deletion allele might be a risk factor in certain subgroups [10]. In line with these findings, our study observed that the frequency of the DD genotype was approximately twice as high in the breast cancer group compared to the control group, suggesting a potential contribution of the D allele to breast cancer susceptibility.

In a nested case-control study conducted among Chinese women in Singapore, individuals with low-activity A and I alleles were found to have a statistically significant reduction in breast cancer risk compared to those with high-activity genotypes [4]. Our study, conducted independently of these findings, also revealed that the frequency of the D allele was higher in the breast cancer group than in the control group, consistent with the hypothesis that increased ACE activity may facilitate tumor progression through enhanced angiogenesis and proliferative signaling.

Comprehensive meta-analyses have continued to report conflicting findings. Ruiter et al. emphasized the inconsistencies in the literature, noting that while some studies demonstrated a significant association between ACE I/D polymorphism and cancer risk, including breast cancer, others reported no such relationship [1]. The authors proposed that these discrepancies could stem from heterogeneity in study design, differences in sample size, population genetics, genotyping techniques, and even unaccounted confounding factors such as hormonal status or environmental exposures. Another large-scale meta-analysis involving 10,405 subjects similarly concluded that no definitive association could be

established and stressed the urgent need for well-designed, multicenter, and ethnically stratified studies to clarify the clinical significance of the ACE I/D polymorphism [11].

More recent studies have aimed to address these discrepancies with population-specific findings. Essobky et al. found no significant difference in overall genotype frequencies between patients and controls in Egyptian women but reported that the ID genotype was associated with HER2 positivity and aggressive tumor characteristics [12]. Similarly, Alves Corrêa et al. reported that the ID genotype appeared to confer a protective effect in Brazilian women, lowering breast cancer risk under certain models [13]. Sharique et al. reported no overall association in North Indian women, but observed a trend toward increased DD genotype frequency among patients with advanced tumor stage [14]. Sa'id et al. demonstrated a moderate but non-significant increase in breast cancer risk among North African women carrying the D allele, though the study was limited by small sample size [15]. Sun et al. confirmed that the ACE I/D polymorphism might have a marginal effect on breast cancer susceptibility in Asian women under allele and homozygous models [16]. Van der Knaap et al. in the Rotterdam study highlighted that the effect of ACE genotype may also be modulated by the use of renin-angiotensin system inhibitors, an important consideration for future pharmacogenomic research [17]. Furthermore, Ladd et al. and Haiman et al. showed population-specific differences, emphasizing that genotype–phenotype correlations may vary across ethnic groups [9, 10]. Koh et al. similarly found a modest association between ACE polymorphism and breast cancer risk in Chinese women, underscoring the role of ethnic stratification in interpreting results [4].

In our study, we investigated the association between ACE (I/D) gene polymorphism and breast cancer risk specifically within the Turkish population. While no statistically significant difference was detected between breast cancer patients and controls at the $p<0.05$ level, the observed higher frequency of the D allele in the tumor group is noteworthy and may reflect a biologically relevant trend. This finding underscores the possible role of the D allele in predisposing individuals to breast cancer, even in the absence of statistical significance in small sample sizes.
Taken together, the available evidence suggests that the association between ACE I/D polymorphism and breast cancer risk is complex and likely influenced by multiple genetic and environmental factors. Some studies point toward a potential risk-modifying effect of the D allele, particularly in certain ethnic groups, whereas others fail to confirm this association. This inconsistency highlights the need for larger, adequately powered studies with careful control for confounders and the use of standardized genotyping methods. Future research should also explore gene-gene and gene-environment interactions, as well as functional studies that elucidate the mechanistic pathways linking ACE activity to breast cancer pathogenesis. Such work could eventually help determine whether ACE I/D polymorphism could serve as a predictive biomarker or therapeutic target in breast cancer management.

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