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# Association between the polymorphisms of angiotensin converting enzyme (Peptidyl-Dipeptidase A) INDEL mutation (I/D) and Angiotensin II type I receptor (A1166C) and breast cancer among post menopausal Egyptian females



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## KEYWORDS

Breast neoplasm;  
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**Abstract** Renin angiotensin system (RAS) is involved in the regulation of cardiovascular homeostasis. Angiotensin (Ang II) is converted from angiotensin I via angiotensin converting enzyme (ACE). Ang II exerts its effects by binding to two types of receptors; AT<sub>1</sub>R and AT<sub>2</sub>R. Ang II effect on AT<sub>1</sub>R promotes proliferation, angiogenesis and metastasis in breast tissues. ACE (I/D) polymorphism is an insertion/deletion of a 287 bp DNA fragment within intron 16 of ACE gene. A1166C is a single nucleotide polymorphism (SNP) in the 3'-UTR of AT<sub>1</sub>R gene. Both (D) and (C) alleles were found to be related to RAS overactivation.

**Subjects and methods:** One hundred and twenty postmenopausal Egyptian females were included in the present study and were divided into control group (fifty apparently healthy women) and patients group (seventy breast cancer patients). Detailed history taking was done with stress on age, family history, menstrual, obstetric, medical and drug history. Physical examination including body mass index calculation was done. Histopathological examination was done for tumor grading and staging. Detection of ACE gene (I/D) polymorphism by PCR and AT<sub>1</sub>R A1166C SNP using PCR/RFLP were done.

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**Results:** A statistically significant difference in AT<sub>1</sub>R A1166C SNP genotype frequencies was found among the studied groups. The patients group showed higher frequency of “CC” (2.9% vs 0%) and “AC” (44.3% vs 24%) and lower frequency of “AA” genotype (52.9% vs 76%) than controls. The patients also showed significant higher frequency of allele “C” (25% vs 12%) which was associated with increased breast cancer risk with an Odds ratio of 2.4444 (95% CI: 1.1967–4.9931). Testing the dominant model of inheritance revealed a statistically higher frequency of exposed genotypes “AC and CC” among the patients group (47.1% vs 24%, respectively;  $p = 0.013$ ) with substantial increase in breast cancer risk among the exposed genotypes with an Odds ratio of 2.8243 (95% CI: 1.2679–6.2913). The present study demonstrated that (AC and CC) genotypes of AT<sub>1</sub>R A1166C SNP and increased BMI can be considered as predictors for breast cancer risk among post menopausal Egyptian females. Results also revealed that A1166C SNP of AT<sub>1</sub>R gene and ACE/ID polymorphism could not be considered as predictors for breast cancer prognosis.

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## 1. Introduction

The Renin angiotensin system (RAS) is critically involved in the physiological regulation of blood pressure, volume homeostasis and tissue perfusion. It plays an integral role in the pathogenesis of hypertension and other cardiovascular diseases.<sup>1,2</sup> RAS is now well recognized as a dual vasoactive system, acting as both a circulating endocrine system and a local tissue system.<sup>3,4</sup>

Angiotensin converting enzyme (ACE or Peptidyl-Dipeptidase A) is a zinc dependant dipeptidyl carboxypeptidase that cleaves a dipeptide from the carboxyl terminus of the decapeptide Ang I to form Ang II. In addition ACE metabolizes a number of other peptides, including the vasodilator peptide bradykinin, to inactive metabolites. Thus, functionally ACE has a dual enzymatic action resulting in increased vasoconstriction and decreased vasodilation.<sup>5</sup>

Ang II is a potent vasoconstrictor which acts by binding to three recognized angiotensin receptor subtypes, angiotensin receptor type 1 (AT<sub>1</sub>R) and the angiotensin receptor type 2 (AT<sub>2</sub>R), both are structurally similar, and angiotensin receptor type 4 (AT<sub>4</sub>R) which is different.<sup>6,7</sup> Binding of Ang II to AT<sub>1</sub>R stimulates vasoconstriction, release of aldosterone, angiogenesis, cell growth and proliferation while its binding to AT<sub>2</sub>R causes growth inhibition, apoptosis and vasodilatation.<sup>7,8</sup>

In human, the gene encoding ACE is located on the long arm of chromosome 17 (17q23). The gene is 21 kilo bases (kb) long and comprises 26 exons and 25 introns. The most popular ACE gene polymorphism is an insertion/deletion (I/D) of a 287-base-pair DNA fragment within the intron 16 of the ACE gene (NCBI Ref. SNP ID: rs1799752). This polymorphism was found to be linked to the ACE activity level which is doubled in homozygous deletion carriers (DD) when compared to II carries, while ID carriers show intermediate activity.<sup>9–11</sup>

The human AT<sub>1</sub>R gene is mapped to chromosome 3q21–q25 and spans more than 55 kb of genomic DNA. A1166C (NCBI Ref. SNP ID: rs5186) is a single nucleotide polymorphism (SNP) in which there is an A/C transversion at position 1166 in the 3′ untranslated region (3′-UTR) of AT<sub>1</sub>R gene. It has been found that the A1166C SNP occurs in a *cis*-regulatory site in the 3′-UTR of AT<sub>1</sub>R gene which after transcription is recognized by a specific microRNA (miR-155).<sup>9</sup>

Micro RNAs (miRNAs) are a class of small endogenous non coding single stranded RNAs (19–24 nucleotides) that are involved in post transcriptional gene regulation. Micro RNAs regulate gene expression through binding to their target messenger RNAs (mRNAs) in the 3′-UTR, by a partial base-pairing mechanism. This binding would result in either inhibition of translation or induction of target mRNA degradation.<sup>11,12</sup> In AT<sub>1</sub>R gene; the A1166C SNP is considered one of the miRSNPs (SNP in a micro RNA target site) that alter a DNA sequence which when transcribed will form a part of a *cis*-regulatory site located in the AT<sub>1</sub>R mRNA 3′-UTR, at a site where miR-155 is known to interact. When A allele is present in this regulatory site, there will be at least seven consecutive base pair regions of complementarity between the 5′ end of miR-155 and the AT<sub>1</sub>R mRNA target site, thus enabling miR-155 to interact with this regulatory site thus inhibiting AT<sub>1</sub>R mRNA translation. On the other hand, when the C-allele is present, base-pairing complementarity is interrupted, and the ability of miR-155 to interact with the *cis*-regulatory site is decreased. As a consequence, miR-155 can no longer attenuate translation, resulting in increased AT<sub>1</sub>R densities leading to an increase in RAS activation.<sup>9,11,13</sup>

The RAS, in particular the AT<sub>1</sub>R, is often up-regulated during the progression from normal to malignant phenotypes, indicating a possible correlation between the RAS and tumor progression.<sup>14,15</sup> The RAS plays a role in modulation of angiogenesis, cellular proliferation, immune responses, inflammation and extracellular matrix formation. Manipulation of the RAS may, therefore, provide a safe and inexpensive anticancer strategy.<sup>16–18</sup>

In Egypt, breast cancer represents 24% of total cancer cases (37% in women and 0.8% in men) according to latest records of Egypt National Cancer Institute (NCI) series of 55,740 patients between 2002 and 2007.<sup>17</sup> The three strongest prognostic determinants in operable breast cancer are lymph node (LN) stage, primary tumor size, and tumor histologic grade, they were combined in an index termed Nottingham prognostic index (NPI).<sup>18</sup>

It has been suggested that a positive correlation might exist between polymorphisms of genes that code for proteins of the RAS and the risk of developing breast cancer.<sup>19,9</sup> So far, there are no data available in the literature regarding the association of AT<sub>1</sub>R receptor (A1166C) and ACE gene (I/D)

polymorphisms in Egyptian women with breast cancer, the aim of the study is assessment of these polymorphisms in Egyptian females with breast cancer.

**Aim of the work:** Studying any possible association between the polymorphisms of angiotensin converting enzyme (I/D) and angiotensin II type I receptor (A1166C) and breast cancer among post menopausal Egyptian females.

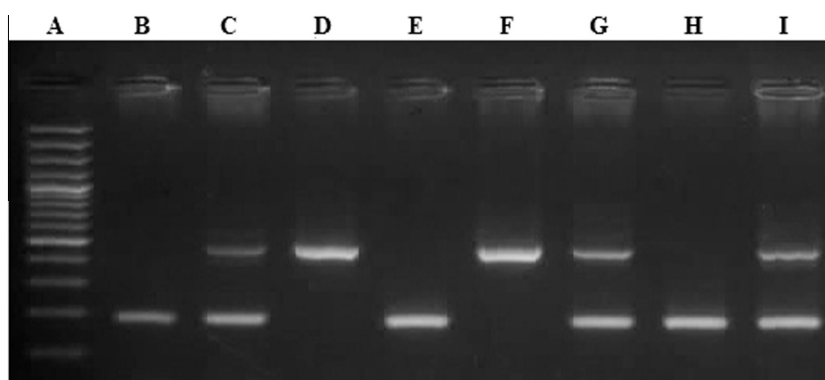
**Subjects and methods:** After the acceptance of the Ethics Committee of the Medical Research Institute (MRI), one hundred and twenty Egyptian females were recruited from the outpatient clinic of the MRI hospital, they were divided as follows: *Group I (control group)*: It included fifty apparently healthy postmenopausal Egyptian females without any family history of breast cancer. *Group II (patients group)*: it included seventy postmenopausal Egyptian females with breast cancer with positive family history. **Exclusion criteria:** patients taking ACE inhibitors (ACEIs), angiotensin II receptor blockers (ARBs) or any drugs that affect ACE or angiotensin II level, patients with history or clinical evidence of any other malignancy and patients with liver, renal and lung diseases.

An informed consent was taken from all subjects included in this study before its start.

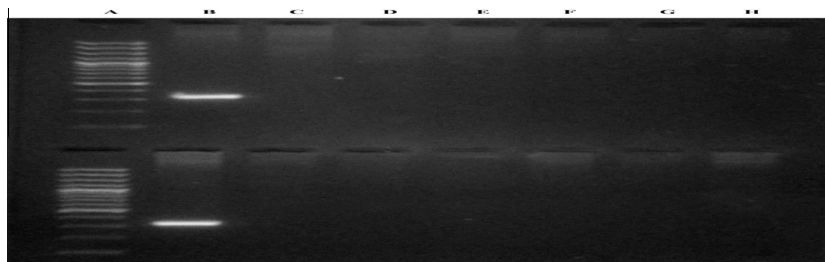
**All studied subjects were subjected to the following:** Detailed history taking with special stress on age, age of onset of breast cancer, family history, menstrual and obstetric history including age of menarche, age of menopause, parity, medical history, drug history especially intake of antihypertensive drugs, oral contraceptive pills (OCPs) and hormonal replacement therapy (HRT). Thorough physical examination including

estimation of body mass index (BMI). Histopathological examination of the breast tissues was done for tumor grading and histological staging.<sup>20,21</sup>

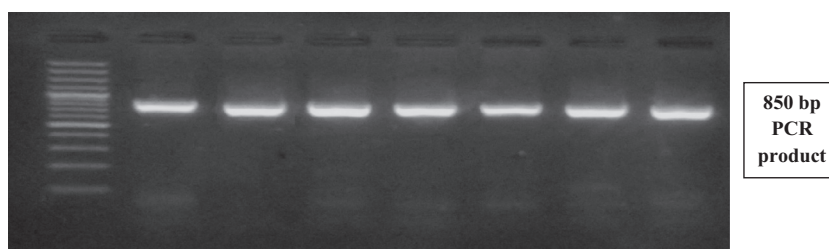
**Laboratory investigations:** After 10 h fasting; five milliliters of venous whole blood was withdrawn from each subject and the following parameters were performed: serum glucose, urea and creatinine concentrations, serum activity of alkaline phosphatase, alanine amino transferase and aspartate amino transferase were conducted on Olympus AU400 clinical chemistry analyzer (Beckman Coulter Inc.).<sup>22</sup> Serum CA 15.3 was done on IMMULITE 1000, using a two-step sequential chemiluminescent immunometric assay<sup>23</sup> and molecular studies were done which included: DNA extraction from peripheral blood leucocytes, followed by detection of the presence of the (I) and (D) alleles in the ACE gene by Polymerase chain reaction (PCR) amplification using specific primers (Forward primer: 5'-CTG GAG ACC ACT CCC ATC CTT TCT-3', Reverse primer: 5'-GAT GTG GCC ATC ACA TTC GTC AGA T-3') followed by agarose gel electrophoresis for the PCR product see (Fig. 1). All samples found to be (DD) after amplification with the conventional primers, were reamplified using allele specific primer pair which recognized insertion specific sequences (Forward primer: 5'-TGG GAC CAC AGC GCC CGC CAC TAC-3', Reverse primer: 5'-TCG CCA GCC CTC CCA TGC CCA TAA-3'). Allele (Insertion) Specific PCR was done to avoid mistyping of the (I) allele as (D) allele in (ID) heterozygous carriers see (Fig. 2). Detection of AT<sub>1</sub>R (A1166C) gene polymorphism using polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP)



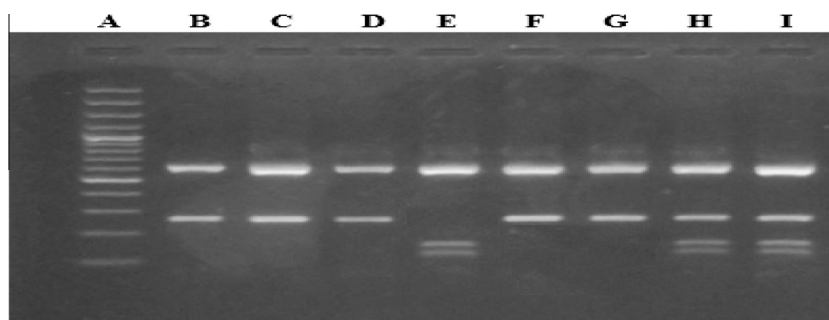
**Figure 1** Agarose gel electrophoresis showing (I/D) polymorphisms of the ACE gene. (D) and (I) alleles were identified by the presence of 190-bp and 490-bp fragments, respectively. Lane A: 100–3000 DNA ladder, Lane B: (DD), Lane C: (ID), Lane D: (II), Lane E: (DD), Lane F: (II), Lane G: (ID), Lane H: (DD), Lane I: (ID).



**Figure 2** Allele specific amplification using insertion-specific sequences. The (I) allele produced a 335 bp amplicon. Lane A (upper and lower rows): 100–3000 bp DNA ladder. Lane B (upper row): II (positive control). Lane B (lower row): ID mistyped as DD. Lanes C–H (upper and lower rows): correctly typed DD.



**Figure 3** PCR product on 2% agarose gel at 850 bp with (100–3000 bp) DNA ladder marker.



**Figure 4** Dde I restriction digestion of the PCR product Homozygote (CC) produced three bands (600, 140 and 110 bp long) homozygote (AA) produced two bands (600 and 250 bp long). Heterozygote (AC) produced all four bands. Lane A: 100–3000 bp DNA ladder. Lanes B–D, F, G: homozygous (AA). Lane E: homozygous (CC). Lanes H and I: heterozygous (AC).

technique was done as follows: PCR amplification using specific primers (Forward primer: 5'-AAT GCT TGT AGC CAA AGT CAC CT-3', Reverse primer: 5'-GGC TTT GCT TTG TCT TGT TG-3') see (Fig. 3), restriction digestion of PCR products using *Dde-I* enzyme and agarose gel electrophoresis of digested PCR products to detect A1166C gene polymorphism see (Fig. 4).

## 2. Statistical analysis<sup>24</sup>

Statistical analysis was done using SPSS program version 20 (Statistical Package of social sciences, Chicago, USA).<sup>25</sup> For quantitative variables: D'Agostino – Pearson  $K$ -squared test for normality was used to test for the degree of deviation from normal distribution across all quantitative variables in all groups and subgroups. For qualitative variables: The data of the qualitative variables were summarized in the form of frequency or percentages. The Chi-Square test ( $\chi^2$  test) with a Monte Carlo estimate of the exact  $p$ -value, was used to compare proportions of nominal clinical data variables between groups for 2 by 3 table or more (not for  $2 \times 2$  tables). Chi-square test was performed only if at least 80% of the cells have an expected frequency of 5 or greater, and no cell has an expected frequency smaller than 1. For  $2 \times 2$  tables, Fisher's Exact Test was used to compare proportions of nominal clinical data variables. Fisher's Exact Test can only be applied to 2 by 2 tables whatever the cell values are. For 2 or more by 3 or more tables with less than 80% of the cells having an expected frequency of  $\geq 5$ , or one or more cells having an expected frequency  $< 1$ , neither the Chi-square test nor the normal Fisher's exact test could be used. In this situation the Freeman–Halton<sup>26</sup> extension of the Fisher's exact probability test (Fisher–Freeman–Halton's test) was used instead.  $p$ -Values

of less than 0.05 was considered a statistically significant difference.

Testing deviation from the HWE is generally performed using Pearson's Chi-Squared goodness of fit test, using the observed genotype frequencies obtained from the data and the expected genotype frequencies obtained using the HW equations.<sup>27</sup>

*Odds ratio and risk assessment.*<sup>28</sup> To measure the effect or the impact of A1166C AT<sub>1</sub>R SNP and ACE I/D genotypes or alleles on the risk of developing breast cancer.

*Logistic regression analysis:* Used to determine the relationship between predictor variables and a dichotomously coded dependent variable.<sup>29</sup>

## 3. Results

A statistically significant difference ( $p = 0.0211^*$ ) in the AT<sub>1</sub>R A1166C SNP genotype frequencies between breast cancer patients and control groups was found. The breast cancer patients had a higher frequency of the homo-mutant genotype "CC" than controls (2.9% vs 0%), a higher frequency of the hetero-mutant genotype "AC" than controls (44.3% vs 24%) and a lower frequency of the homo-wild genotype "AA" than controls (52.9% vs 76%) (Table 1).

There was a statistically significant ( $p = 0.0134^*$ ) difference in the A1166C AT<sub>1</sub>R SNP allele frequencies between breast cancer patients and controls, while the patients had statistically significant higher frequency of the allele "C" than controls (25% vs 12%) and allele C was associated with a statistically increased breast cancer risk with an Odds ratio of 2.4444 (95% CI: 1.1967–4.9931) (Table 2).

Testing the recessive model was not applicable due to the low number of CC genotypes; also the Odds ratio cannot be

**Table 1** Comparison of AT<sub>1</sub>R A1166C SNP genotype frequencies among the studied groups.

| A1166C AT <sub>1</sub> R SNP genotype | Control group (n = 50) | Patients group (n = 70) | Exact p-value  |
|---------------------------------------|------------------------|-------------------------|----------------|
| AA (n = 75; 62.5%)                    | 38 (76%)               | 37 (52.9%)              | p = 0.021108 * |
| AC (n = 43; 35.8%)                    | 12 (24%)               | 31 (44.3%)              |                |
| CC (n = 2; 1.7%)                      | 0 (0%)                 | 2 (2.9%)                |                |
| Total (n = 120; 100%)                 | 50 (100%)              | 70 (100%)               |                |

**Table 2** Comparison of AT<sub>1</sub>R A1166C SNP allele frequencies among the studied groups.

| A1166C alleles | Control group (n = 50) | Patients group (n = 70) | Total       | Exact p-value | Odds ratio (95% CI)    |
|----------------|------------------------|-------------------------|-------------|---------------|------------------------|
| Allele A       | 88 (88%)               | 105 (75%)               | 193 (80.4%) | p = 0.0134 *  | 2.4444 (1.1967–4.9931) |
| Allele C       | 12 (12%)               | 35 (25%)                | 47 (19.6%)  |               |                        |
| Total          | 100 (100%)             | 140 (100%)              | 240 (100%)  |               |                        |

**Table 3** Assessment of the risk of developing breast cancer according to AT<sub>1</sub>R + A1166C SNP genotypes in different models of inheritance.

| Assumed model of inheritance | Model (unexposed vs exposed) | Genotype  | Control group n = 50 (100%) | Patients group n = 70 (100%) | Exact p-value | Odd's ratio (95% CI)   |
|------------------------------|------------------------------|-----------|-----------------------------|------------------------------|---------------|------------------------|
| <sup>a</sup> Recessive       | (AA and AC) vs CC            | AA and AC | 50 (100%)                   | 68 (97.1%)                   | –             | –                      |
|                              |                              | CC        | 0.0 (0.00%)                 | 2 (2.9%)                     |               |                        |
| <sup>b</sup> Dominant        | AA vs (AC and CC)            | AA        | 38 (76%)                    | 37 (52.9%)                   | 0.013*        | 2.8243 (1.2679–6.2913) |
|                              |                              | AC and CC | 12 (24%)                    | 33 (47.1%)                   |               |                        |

estimated because of the absence of CC genotype among the control subjects.

Testing the dominant model of inheritance revealed a statistically higher frequency of exposed genotypes “AC and CC” among the breast cancer patients group when compared to control group (47.1% vs 24%, respectively; p = 0.013) with substantial increase in breast cancer risk among the exposed group “AC and CC” with an Odds ratio of 2.8243 (95% CI: 1.2679–6.2913) when compared to unexposed group “AA” (Table 3). Only BMI and A1166C AT<sub>1</sub>R SNP genotype were left as significant predictors in this logistic regression model. The Wald criterion demonstrated that both BMI and A1166C AT<sub>1</sub>R SNP genotype made a significant contribution to the prediction of breast cancer risk (p-value = 0.001 and 0.009, respectively) (Table 4).

No statistically significant difference was found (p = 0.940) in the ACE I/D genotype frequencies between the studied groups (Table 5). No statistically significant difference (Exact p-value = 0.893, Odd's ratio = 0.9353 with 95% confidence interval: 0.5507–1.5886) in the ACE I/D allele frequencies was found among the studied groups (Table 6).

**4. Discussion**

Breast cancer accounts for 18% of all female cancers worldwide and it is one of the main causes of global health burden. Several environmental, anthropometric, and genetic factors could contribute to increased risk of breast cancer. A number of genetic variants have been identified to be potentially associated with breast cancer risk.<sup>30</sup>

**Table 4** Variables included in the final logistic regression model with breast cancer risk as the dependent variable.

| Variable  | Wald   | p-Value | Exp (B) | 95% CI for Exp (B) |
|---|--------|---------|---------|--------------------|
| Body mass index (BMI)                                       | 11.146 | 0.001   | 1.265   | (1.102–1.452)      |
| A1166C AT <sub>1</sub> R SNP genotype (AC and CC versus AA) | 6.875  | 0.009   | 3.419   | (1.364–8.569)      |

**Table 5** Comparison of ACE I/D genotype frequencies among the studied groups.

| ACE I/D genotype      | Control group (n = 50) | Patients group (n = 70) | Exact P-value |
|-----------------------|------------------------|-------------------------|---------------|
| II (n = 21; 17.5%)    | 8 (16%)                | 13 (18.6%)              | p = 0.940     |
| ID (n = 49; 40.8%)    | 21 (42%)               | 28 (40%)                |               |
| DD (n = 50; 41.7%)    | 21 (42%)               | 29 (41.4%)              |               |
| Total (n = 120; 100%) | 50 (100%)              | 70 (100%)               |               |

**Table 6** Comparison of ACE I/D allele frequencies among the studied groups.

| ACE I/D       | Control group ( <i>n</i> = 50) | Patients group ( <i>n</i> = 70) | Total      | Exact <i>P</i> -value | Odds ratio (95% CI)    |
|---------------|--------------------------------|---------------------------------|------------|-----------------------|------------------------|
| Insertion (I) | 37 (37%)                       | 54 (39%)                        | 91 (38%)   | 0.893                 | 0.9353 (0.5507–1.5886) |
| Deletion (D)  | 63 (63%)                       | 86 (61%)                        | 149 (62%)  |                       |                        |
| Total         | 100 (100%)                     | 140 (100%)                      | 240 (100%) |                       |                        |

There is now increasing evidence that RAS may have local tissue actions involving angiogenesis, cellular proliferation, apoptosis and inflammation. A polymorphic variant composed of an insertion (I) or a deletion (D) of a 287 base pair insert in intron 16 (rs: 1799752) has been identified to be associated with altered concentration of ACE.<sup>31</sup> The DD genotype is associated with the highest serum levels of the enzyme, the ID genotype with the intermediate levels and the II genotype with the lowest levels.<sup>32,33</sup>

A1166C is a single nucleotide polymorphism (SNP) in which there is an A to C transversion at position 1166 in the 3' untranslated region of the AT<sub>1</sub>R gene. When the C-allele is transcribed the ability of micro-RNA 155 (miR-155) to attenuate AT<sub>1</sub>R mRNA translation is interrupted, resulting in an overall increase in AT<sub>1</sub>R density and thus increasing the biological actions of Ang II resulting in RAS over activation.<sup>34,37</sup>

In the present study, the association between ACE I/D and AT<sub>1</sub>R A1166C SNP and breast cancer was studied in a sample of one hundred and twenty Egyptian females divided into two groups; The control group (Group I) included fifty apparently healthy postmenopausal Egyptian females without family history of breast cancer; their mean age was 62.74 ± 5.47 years. The patient group (Group II) included seventy postmenopausal Egyptian females with breast cancer; their mean age was 52.1 ± 5.79 years.

Comparing the genotype frequencies of ACE (I/D) polymorphism among the studied groups revealed no statistically significant difference (Table 5; *p* = 0.940) between the control group (II = 16%, ID = 42%, DD = 42%) and breast cancer patients group (II = 18.6%, ID = 40%, DD = 41.4%). Furthermore, there was also no statistically significant difference in the allelic frequencies of ACE (I/D) polymorphism between control group ("I" allele = 37%, "D" allele = 63%) and breast cancer patients group ("I" allele = 39%, "D" allele = 61%) (Table 6; *p* = 0.893, OR = 0.9353; 95% CI: 0.5507–1.5886). The genotype distribution as well as allele frequencies of ACE (I/D) polymorphism were both found to be in agreement with Hardy–Weinberg equilibrium. Several studies tried to explore the association between ACE ID polymorphism and breast cancer risk; however they revealed conflicting results. Some studies<sup>30,35</sup> reported that D allele was positively associated with breast cancer risk; another study<sup>36</sup> reported that D allele was negatively associated with breast cancer risk; and others<sup>37</sup> (in agreement with the present study) found no significant difference suggesting that ACE I/D polymorphism may not be a good predictor for breast cancer risk.

Koh et al.<sup>35</sup> first studied both I/D and A240T polymorphisms of the ACE gene in association with breast cancer, they found that women with both A and I alleles had a statistically significant reduction of breast cancer risk compared with those carrying either the TT and/or DD genotypes (the high-activity genotype) (OR: 0.46; 95% CI: 0.27–0.81). González-Zuloeta Ladd et al.<sup>30</sup> conducted a prospective cohort study to evaluate

relationship of the ACE I/D polymorphism with breast cancer risk in 4117 Caucasian postmenopausal women. They found that DD carriers showed a significantly increased risk of developing breast cancer when compared with the II carriers (OR: 1.86; 95% CI: 1.06–3.27). This association remained after performing logistic regression analysis to adjust for other risk factors, including body mass index, age at menarche, age at menopause, hormone replacement therapy, and hypertension. In agreement with the previous studies; Van der Knaap et al.<sup>38</sup> found that carriers of the high activity genotype DD had an increased risk of breast cancer compared with low-activity II/ID genotype carriers (hazard ratio [HR]: 1.47; 95% CI: 1.05–2.04), but no association was demonstrated for other cancers.

On the other hand Haiman et al.<sup>36</sup> studied ACE I/D polymorphisms in relation to breast cancer risk among different ethnic groups. They found that D allele was reversely associated with breast cancer risk. (OR: 1.30; 95% CI: 1.05–1.61), although associations were not entirely consistent across ethnic groups suggesting that D allele may be a marker of low ACE levels in some populations, but not all populations.

In agreement with the present study; Sun et al.<sup>37</sup> conducted a recent meta analysis which included four studies (cases: 1422; controls: 3044), they studied the association between ACE (I/D) polymorphism and breast cancer. The results of this meta analysis showed that there was no significant difference in genotype distribution (DD, ID or II) between breast cancer patients and controls. They suggested that ACE I/D polymorphism may not be a genetic risk factor for breast cancer.

These discrepancies in results might be due to insufficient statistical power and lack of adjustment for other potential breast cancer confounding factors in some studies, recruitment procedures of the study population, genetic heterogeneity and differences in linkage disequilibrium between different populations as well as environmental backgrounds. Another reason could be the differences in population characteristics (e.g., use of ACE inhibitors and green tea intake<sup>39</sup> could influence the association between RAS genes and breast carcinogenesis), these differences in patient characteristics are likely to influence their phenotypes and may have an impact on the study results. Using different methods and techniques with different performance characteristics (e.g., insertion specific amplification to avoid ID mistyping as DD was not done in all studies) could be another possible reason for result inconsistency.

As for A1166C SNP of AT<sub>1</sub>R gene, comparing the genotype frequencies among the studied groups (Table 1) revealed a statistically significant difference (*p*-value = 0.0211\*) between control group and breast cancer patients group, where the breast cancer patients have a higher frequency of the homozygous mutant genotype (CC) than controls (2.9% vs 0%), a higher frequency of the heterozygous mutant genotype (AC) than controls (44.3% vs 24%) and a lower frequency of the homozygous wild genotype (AA) than controls (52.9% vs 76%). Furthermore, there was also a statistically significant

difference ( $p = 0.0134$ ) in the allelic frequencies of A1166C SNP of AT<sub>1</sub>R gene between control group and breast cancer patients group, where the breast cancer patients had a higher frequency of the mutant allele (C) than controls, and a lower frequency of the wild allele (A) than controls (OR 2.4444; 95% CI: 1.0616–4.0233) (Table 2). From these results it could be concluded that the mutant allele (C) was associated with a statistically significant increased risk of developing breast cancer. The genotype distribution as well as allele frequencies of A1166C SNP of AT<sub>1</sub>R gene were both found to be in agreement with Hardy–Weinberg equilibrium.

Assuming a recessive model of inheritance for AT<sub>1</sub>R A1166C SNP; the exposed group is only subjects with the (CC) genotype while the unexposed group is subjects with both (AC and AA) genotypes combined. Unfortunately, in the present study the Odds ratio involving the (CC) genotype alone could not be estimated because of the absence of this genotype among the control subjects. Accordingly assessing the risk in the recessive model was not possible. Results of the present study revealed also a statistically higher frequency of exposed genotypes “AC and CC” among the breast cancer patients group when compared to control group ( $p = 0.013$ ) with substantial increase in breast cancer risk among the exposed group (AC and CC) with an OR of 2.8243 (95% CI: 1.2679–6.2913) when compared to unexposed group (AA) (Table 3). This finding remains after adjustment for other breast cancer confounding factors, the variables included in the final logistic regression model with breast cancer risk as the dependent variable were BMI ( $p$ -value: 0.001, Exp (B): 1.265, 95% CI: 1.102–1.452) and A1166C AT<sub>1</sub>R SNP genotype (AC and CC vs AA) ( $p$ -value: 0.009, Exp (B): 3.419, 95% CI: 1.364–8.569) (Table 4).

The findings of the present study could be explained by the ability of AT<sub>1</sub>R A1166C SNP to cause RAS overactivation. Since A1166C polymorphism occurs in the 3'-UTR of the human AT<sub>1</sub>R gene, the biological mechanism by which this SNP could possibly lead to RAS overactivation has been always questionable. Martin et al.<sup>11</sup> and Sethupathy et al.<sup>12</sup> demonstrated that A1166C SNP is one of the miRSNPs that alter a DNA sequence which when transcribed will form a part of a *cis*-regulatory site located in the AT<sub>1</sub>R mRNA 3'-UTR, at a site where miRNA “miR-155” is known to interact. When the (C) allele is present, base-pairing complementarity is interrupted, and the ability of miR-155 to interact with this *cis*-regulatory site is decreased. As a consequence, miR-155 can no longer attenuate translation, resulting in increased AT<sub>1</sub>R densities leading to an increase in RAS activation.<sup>40</sup> Increased AT<sub>1</sub>R mRNA transcription was observed in breast cancer cells when compared to normal cells. This will subsequently lead to enhanced tumorigenic actions of Ang II on breast cells including angiogenesis, cell proliferation, and inflammation. Since AT<sub>1</sub>R A1166C SNP is known to increase AT<sub>1</sub>R mRNA expression, therefore this SNP might be related to increased AT<sub>1</sub>R expression in breast cancer cells.<sup>41,42</sup>

So far, only limited studies have evaluated the association between AT<sub>1</sub>R A1166C SNP and breast cancer risk, yet they also yielded inconsistent results. Alves Corrêa et al.<sup>43</sup> and Namazi et al.<sup>33</sup> suggested that the A1166C polymorphism was not associated with breast cancer risk ( $p$ -values: 0.114 and 0.86, respectively). In contrast, Mendizábal-Ruiz et al.<sup>44</sup> found that the frequency of (A) allele was higher in breast cancer patients group when compared to controls ( $p = 0.036$ ) and that the (C) allele carriers had reduced risk of breast cancer

(OR: 0.53,  $p$ -value = 0.0356). Recently; Xi et al.<sup>45</sup> evaluated the three published studies (235 cases and 601 controls). Significant association between AT<sub>1</sub>R A1166C SNP and breast cancer risk was observed for (AC) versus (AA) and dominant model. However, after excluding one study that was not in Hardy–Weinberg equilibrium (by Alves Corrêa et al.<sup>43</sup>, this association disappeared while a marginally significant association was observed for CC versus AA (OR = 0.31, 95% CI 0.10–0.99). Since publication bias was not assessed for these findings; they recommended further studies to explore the association between this SNP and breast cancer risk.

From the previously mentioned studies, only limited data are available regarding the association between AT<sub>1</sub>R A1166C SNP and breast cancer risk. Lack of consistency in the findings, limited sample size, insufficient statistical power for one study as well as paucity of explanations for the observed associations could be noticed. Further large population based studies are still needed to get a conclusive evidence about the association between AT<sub>1</sub>R A1166C SNP and the risk of breast cancer, as well as AT<sub>1</sub>R expression in breast cancer tissues in relation to this SNP genotype.

In conclusion, the present study demonstrated that (AC and CC) genotypes of AT<sub>1</sub>R A1166C SNP together with increased BMI can be considered as significant predictors for breast cancer risk among post menopausal Egyptian Females. Results also revealed that A1166C SNP of AT<sub>1</sub>R gene and ACE/ID polymorphism might not be a good predictor for breast cancer prognosis. Also, pharmacological inhibition of the angiotensin II carcinogenic effect through inhibition of AT<sub>1</sub>R together with weight reduction could be used to delay or prevent the occurrence of breast cancer in post menopausal females, although more detailed trials and experiments are required to confirm this conclusion.

### Conflict of interest

None.

### References

1. Nguyen Dinh Cat A, Touyz RM. A new look at the renin-angiotensin system-focusing on the vascular system. *Peptides* 2011;**32**:2141–50.
2. ARB Trialists Collaboration. Effects of telmisartan, irbesartan, valsartan, candesartan, and losartan on cancers in 15 trials enrolling 138, 769 individuals. *J Hypertens* 2011;**29**:623–35.
3. Bader M, Ganten D. Update on tissue renin-angiotensin systems. *J Mol Med* 2008;**86**:615–21.
4. Zhuo JL, Li XC. New insights and perspectives on intrarenal renin-angiotensin system: focus on intracrine/intracellular angiotensin II. *Peptides* 2011;**32**:1551–65.
5. Carey RM, Siragy HM. Newly recognized components of the renin-angiotensin system: potential roles in cardiovascular and renal regulation. *Endocr Rev* 2003;**24**:261–71.
6. Wright JW, Yamamoto BJ, Harding JW. Angiotensin receptor subtype mediated physiologies and behaviors: new discoveries and clinical targets. *Prog Neurobiol* 2008;**84**:157–81.
7. Muscella A, Greco S, Elia MG, Storelli C, Marsigliante SB. Angiotensin II stimulation of Na<sup>+</sup>/K<sup>+</sup>ATPase activity and cell growth by calcium-independent pathway in MCF-7 breast cancer cells. *J Endocrinol* 2002;**173**:315–23.
8. Nouet S, Nahmias C. Signal transduction from the angiotensin II AT<sub>2</sub> receptor. *Trends Endocrinol Metab* 2000;**11**:1–6.

9. Jeunemaitre X. Genetics of the human renin angiotensin system. *J Mol Med* 2008;**86**:637–41.
10. Sayed Tabatabaei FA, Oostra BA, Isaacs A, van Duijn CM, Wittman JC. ACE polymorphisms. *Circ Res* 2006;**98**:1123–33.
11. Martin MM, Buckenberger JA, Jiang J, Malana GE, Nuovo GJ, Chotani M, et al. The human angiotensin II type 1 receptor +1166 A/C polymorphism attenuates micro RNA-155 binding. *J Biol Chem* 2007;**282**(33):24262–9.
12. Sethupathy P, Borel C, Gagnebin M, Grant GR, Deutsch S, Elton TS, et al. Human microRNA-155 on chromosome 21 differentially interacts with its polymorphic target in the AGTR1 3' untranslated region: a mechanism for functional single-nucleotide polymorphisms related to phenotypes. *Am J Hum Genet* 2007;**81**(2):405–13.
13. Brennecke J, Stark A, Russell RB, Cohen SM. Principles of microRNA-target recognition. *PLoS Biol* 2005;**3**:e85.
14. Smith GR, Missailidis S. Cancer, inflammation and the AT1 and AT2 receptors. *J Inflamm* 2004;**1**(1):3.
15. Tahmasebi M, Barker S, Puddefoot JR, Vinson GP. Localisation of renin-angiotensin system (RAS) components in breast. *Br J Cancer* 2006;**95**(1):67–74.
16. Fujita M, Hayashi I, Yamashina S, Itoman M, Majima M. Blockade of angiotensin AT1a receptor signaling reduces tumor growth, angiogenesis, and metastasis. *Biochem Biophys Res Commun* 2002;**294**(2):441–7.
17. Ali Eldin N. Cancer Statistics 2002–2007, National Cancer Institute, Egypt 2010. Department of Biostatistics and Cancer Epidemiology. Preliminary report.
18. Rakha EA, El-Sayed ME, Lee AH, Elston CW, Grainge MJ, Hodi Z, et al. Prognostic significance of Nottingham histologic grade in invasive breast carcinoma. *J Clin Oncol* 2008;**26**(19):3153–8.
19. Loh JK. Gene polymorphisms in the renin-angiotensin-aldosterone system and breast carcinogenesis: is there a connection? *TSMJ* 2008;**9**:48–51.
20. Rakha EA, Reis-Filho JS, Baehner F, Dabbs DJ, Decker T, Eusebi V, et al. Breast cancer prognostic classification in the molecular era: the role of histological grade. *Breast Cancer Res* 2010;**12**(4):207.
21. National Comprehensive Cancer Network (NCCN) guidelines. Breast Cancer Version 2.2011, pp. ST1-4.
22. Burtis CA, Ashwood ER, Bruns DE. *Tietz text book of clinical chemistry and molecular diagnostics*. 5th ed. Elsevier Saunders Company. St Louis; 2012 pp.718-21, 685–6, 680–4, 578–80 and 575–6, respectively.
23. Slev PR, Rawlins ML, Roberts WL. Performance characteristics of seven automated CA 15-3 assays. *Am J Clin Pathol* 2006;**125**(5):752–7.
24. Daly LE, Bourke GJ. *Interpretation and uses of medical statistics*. 5th ed. Malden, MA: Oxford, Blackwell Science; 2000.
25. Puri BK. *SPSS in practice: an illustrated guide*. 2nd ed. London; New York: Arnold; 2002.
26. Lydersen S, Pradhan V, Senchaudhuri P, Laake P. Choice of test for association in small sample unordered  $r \times c$  tables. *Stat Med* 2007;**26**:4328–43.
27. Rodriguez S, Gaunt TR, Day IN. Hardy–Weinberg equilibrium testing of biological ascertainment for Mendelian randomization studies. *Am J Epidemiol* 2009;**169**(4):505–14.
28. Pieta B, Samulak D, Opala T, Wilczak M, Grodecka-Gazdecka S, Wieznowska-Maczyńska K. Analysis of odds ratio of increased relative risk of developing breast cancer in different groups of women. *Eur J Gynaecol Oncol* 2010;**31**(1):50–4.
29. Peng CJ, Lee KL, Ingersoll GM. An introduction to logistic regression analysis and reporting. *J Educ Res* 2002;**96**(1):3–14.
30. González-Zuloeta Ladd AM, Arias Vázquez A, Sayed-Tabatabaei JW, Coebergh JW, Hofman A, Njajou O, et al. Angiotensin-converting enzyme gene insertion/deletion polymorphism and breast cancer risk. *Cancer Epidemiol Biomarkers Prev* 2005;**14**(9):2143–6.
31. Jia H, Wang B, Yu L, Jiang Z. Association of angiotensin-converting enzyme gene insertion/deletion polymorphism with polycystic ovary syndrome: a meta-analysis. *J Renin Angiotensin Aldosterone Syst* 2013;**14**(3):255–62.
32. Yigit S, Inanir A, Tural S, Ates O. Association of angiotensin converting enzyme (ACE) gene I/D polymorphism and rheumatoid arthritis. *Gene* 2012;**511**(1):106–8.
33. Namazi S, Monabati A, Ardashir-Rouhani-Fard S, Azarpira N. Association of angiotensin I converting enzyme (insertion/deletion) and angiotensin II type 1 receptor (A1166C) polymorphisms with breast cancer prognostic factors in Iranian population. *Mol Carcinog* 2010;**49**(12):1022–30.
34. Zhang K, Zhou B, Zhang L. Association study of angiotensin II type 1 receptor: A1166C (rs5186) polymorphism with coronary heart disease using systematic meta-analysis. *J Renin Angiotensin Aldosterone Syst* 2013;**14**(2):181–8.
35. Koh WP, Yuan JM, Sun CL, van den Berg D, Seow A, Lee HP, et al. Angiotensin I-converting enzyme (ACE) gene polymorphism and breast cancer risk among Chinese women in Singapore. *Cancer Res* 2003;**63**(3):573–8.
36. Haiman CA, Henderson SO, Bretsky P, Kolonel LN, Henderson BE. Genetic variation in angiotensin I-converting enzyme (ACE) and breast cancer risk: the multiethnic cohort. *Cancer Res* 2003;**63**(20):6984–7.
37. Sun M, Liu C, Wei F, Zhong J, Sun Y. Association of angiotensin I converting enzyme insertion/deletion polymorphism with breast cancer: a meta-analysis. *J Renin Angiotensin Aldosterone Syst* 2011;**12**(4):611–6.
38. Van der Knaap R, Siemes C, Coebergh JW, van Duijn CM, Hofman A, Stricker BH. Renin-angiotensin system inhibitors, angiotensin I-converting enzyme gene insertion/deletion polymorphism, and cancer: the Rotterdam study. *Cancer* 2008;**112**(4):748–57.
39. Yuan JM, Koh WP, Sun CL, Lee HP, Yu MC. Green tea intake, ACE gene polymorphism and breast cancer risk among Chinese women in Singapore. *Carcinogenesis* 2005;**26**(8):1389–94.
40. Lim HS, Cho JY, Oh DS, Chung JY, Hong KS, Bae KS, et al. Angiotensin II type 1 receptor 1166A/C polymorphism in association with blood pressure response to exogenous angiotensin II. *Eur J Clin Pharmacol* 2007;**63**:17–26.
41. Jethon A, Pula B, Piotrowska A, Wojnar A, Rys J, Dziegiel P, et al. Angiotensin II type 1 receptor (AT-1R) expression correlates with VEGF-A and VEGF-D expression in invasive ductal breast cancer. *Pathol Oncol Res* 2012;**18**(4):867–73.
42. Rodrigues-Ferreira S, Abdelkarim M, Dillenburg-Pilla P, Luissint A, di-Tommaso A, Deshayes F, et al. Angiotensin II facilitates breast cancer cell migration and metastasis. *PLoS ONE* 2012;**7**(4):e35667.
43. Alves Corrêa SA, Ribeiro de Noronha SM, Nogueira-de-Souza C, Valleta de Carvalho C, Massad Costa AM, Juvenal Linhares J. Association between the angiotensin-converting enzyme (insertion/deletion) and angiotensin II type 1 receptor (A1166C) polymorphisms and breast cancer among Brazilian women. *J Renin Angiotensin Aldosterone Syst* 2009;**10** (1):51–8.
44. Mendizábal-Ruiz AP, Morales J, Castro Martinez X, Gutierrez Rubio SA, Valdez L, Vázquez-Camacho JG, et al. RAS polymorphisms in cancerous and benign breast tissue. *J Renin Angiotensin Aldosterone Syst* 2011;**12**(2):85–92.
45. Xi B, Zeng T, Liu L, Liang Y, Liu W, Hu Y, et al. Association between polymorphisms of the renin-angiotensin system genes and breast cancer risk: a meta-analysis. *Breast Cancer Res Treat* 2011;**130**(2):561–8.