Study of the Link of Angiotensin Converting Enzyme (ACE) Insertion/Deletion (I/D) Polymorphism with Incidence and Pathological Criteria of Breast Cancer

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Abstract: This study was conducted to assess the relationship between angiotensin converting enzyme (ACE) gene insertion/deletion (I/D) polymorphism and Breast cancer among Egyptian women as well as to evaluate its prognostic value. Blood samples from 36 breast cancer cases, and 61 healthy women as a control group were subjected to DNA extraction followed by PCR gene amplification. Obtained results revealed that there was significant association of DD genotype with risk of breast cancer, which was correlated with advanced tumor stage, grade, and distant metastasis. In conclusion, results suggest a possible association of DD genotype and D allele to incidence of poor prognostic criteria of breast cancer.

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1. Introduction

Excluding cancer of the skin, breast cancer is the most common cancer among women, accounting for nearly 1 in 4 cancers diagnosed in US women [**Copeland *et al*., 2009]**. In Egypt, breast cancer is the most common cancer among women, representing 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 **[Ali Eldin 2010].** Poor prognosis of breast cancer has several risk factors determining its aggressiveness including lymph node involvement, tumor size, high histological grade, steroid receptors negativity **[Yaren *et al*., 2006].** Several studies proved the significant linkage of gene polymorphism to the risk of breast cancer **[Shih *et al*., 2002].** BRCA1 or BRCA2 mutations account for only about 50% of familial breast cancer **[Saslow *et al*., 2007].**

The genes involved in breast cancer are expected to be responsible for key processes in cell growth, regulation and cell proliferation including angiogenesis **[Folkman and Shing 1992].** One of the well known angiogenic and growth factor is angiotensin II which is a component of renin angiotensin system (RAS). It has a wide spectrum of target tissues including breast epithelial cells **[Greco *et al*., 2002].** Angiotensin II promotes angiogenesis in cancer cells through up regulation of NADPH oxidase in endothelial cells which stimulate generation of reactive oxygen species (ROS) that participate in vascular endothelial growth factor (VEGF) signaling **[Rueckschloss *et al*., 2002]** which promotes neovascularization in human breast cancer. Angiotensin II is converted to Angiotensin I by angiotensin converting enzyme (ACE) a zinc metalloproteinase which has a variety of functions. ACE inhibition decrease tumor growth in experimental studies including breast cancer cell **[Hii, *et al*., 1998],** and proved to be associated with better prognosis and less incidence of breast cancer **[Van der Knaap *et al*., 2008].** ACE gene is located on chromosome 17q23 it has many polymorphisms, the most commonly studied is I/D polymorphism of 287 bp which is identified in intron 16 that accounts for variability in circulating ACE levels **[Sayed-Tabatabaei *et al*., 2004].**

I/D polymorphism results in genotypes II, ID, and DD. Individuals who are homozygous for D allele exhibit about two folds higher ACE in plasma and tissue levels than II genotype, whereas ID genotype can exhibit intermediate level **[Hubert *et al*., 1991].** On the other hand individuals who are homozygous for I allele have the lowest levels. Because ACE plasma levels correlate with levels of angiotensin II, it was hypothesized that I/D polymorphism may be important in cancer susceptibility. Recent reports showed strong association with risk development of several cancers such as breast, prostate, lung, gall bladder, and gastric cancer **[Namazi *et al*., 2010; Ebert *et al*., 2005; Medeiros *et al*., 2004; Srivastava *et al*., 2010]. Van der Knaap *et al*., 2008** observed a dose dependent protective association between RAS inhibitor use and cancer risk in individuals with DD genotype**.**

The DD genotype has been associated with altered risk of breast cancer **[Gonzalez-Zuloeta Ladd *et al*., 2005]. Koh *et al*., 2003** have reported that women with low activity of ACE genotype exhibited 50% reduction in breast cancer risk as compared within high activity genotype counterparts**.** It has been found that polymorphism of ACE gene is associated with increased risk of breast cancer and significant decrease in cancer free survival in several studies [**Koh *et al*.**,

2005]. Other studies has linked activity and ACE polymorphism to pathological and prognostic criteria in several malignancies, it was correlated with number of lymph node metastasis in gastric cancer patients **[Röcken *et al*., 2005],** DD genotype was linked with higher incidence of advanced stages of prostate cancer **[Medeiros *et al*., 2004]. Yaren *et al*., 2006** has recorded an association between DD genotype and large tumor size of breast cancer in premenopausal women**.**

This investigation aimed to evaluate the distribution of I/D polymorphism of ACE gene in Egyptian patients with breast cancer and to analyze the potential association with pathological criteria of the tumor.

2. Subjects and Methods:

Subjects:

Our study was involving 36 breast cancer Egyptian patients (51.8 years ± 13.5). They were admitted in department of Surgery Ain Shams University hospitals, from May, 2010 to September, 2010. They were staged according to TNM classification of the American Joint Committee on cancer (AJCC) **[Eva Singletary, and James Connolly 2006]** and graded by the Nottingham grading system **[Emad *et al*., 2008].** Their results were compared to 61 healthy volunteer women (45.2years ± 11) as control group. All cases were studied prior to the treatment. None of them was used oral contraceptives, hormones or vitamins. An informed consent was obtained from all included subjects. Blood samples (5 to 10 ml) were withdrawn on Na2-EDTA (final concentration 1mg/m) from all cases for analyses.

DNA extraction:

Genomic DNA was extracted from white blood cell pellets of venous blood samples collected on EDTA tubes by salting out extraction method **[Josef *et al*., 2002]** using wizard genomic DNA extraction kit from Promega. Red blood cell lysis was done by using red cell lysis buffer (20mM Tris-CL pH 7.6) followed by centrifugation. Nuclei lysis was carried by cell lysis buffer (10mM Tris-CL pH 8.0, 1mM EDTA pH 8.0, 0.1% (w/v) SDS) and proteinase K (20mg/ml) followed by centrifugation. Protein was precipitated by protein precipitation solution (60 ml of 5 M potassium acetate, 11.5 ml of glacial acetic acid, 28.5ml of water) followed by centrifugation. Finally DNA was precipitated by isopropanol and then ethanol 70% and rehydrated in TE buffer (pH 7.6) and stored in -20 °C. The DNA purity and concentration were determined by spectrophotometer measurement of absorbance at 260 and 280 nm.

Genetic analysis:

This was followed by polymerase chain reaction (PCR) which was used to determine the frequencies of I/D polymorphisms of the ACE gene **[Mayer *et al*., 2002]** For ACE I/D polymorphism 100 ng of DNA was amplified by polymerase chain reaction (PCR) using Gene Amp PCR system 9700 from Applied Biosystem. The cycling condition was initial denaturation for 3 minutes at 94 ºC followed by 30 cycles of denaturation for 30 seconds at 94 ºC, annealing at 58 ºC for 45 seconds, extension at 68 ºC for 2 minutes, then final extension at 68 ºC for 7 minutes. Each 50 ul of reaction mixture contained 1 µl 50mM magnesium chloride, 5 µl 1% W.1, 2.5 µl DMSO, 5 µl 2mM dNTP, polymerization mix, 0.2 µl 10x Taq DNA polymerase and 100ng of primers. The primers used were: sense- 5'GATGTGGCCATCACATTCGTCAGAT3', and antisense- 5'CTGGAGACCACTCCCATCCTTTCT3'. The reaction products were electrophoresed on 2% agarose gel and stained with ethidium bromide under ultraviolet light. Preferential amplification of the smaller 190 bp deletion allele (D) in ID heterozygote has led to their mistyping as DD homozygote were retyped using an I specific sense primer 5' TTTGAGACGGAGTCTCGCTC3' with then subjected to 94 ºC for 3 minutes followed by 35 cycles denaturation for 30 seconds at 93 ºC, annealing at 67 ºC for 30 seconds and extension at 72 ºC for 30 seconds, and final extension at 72 ºC for 10 minutes the products were separated on 1.5% agarose gel.

Statistical analysis

Statistical analysis was carried out using SPSS statistical package for social sciences (version 11.5, SSPS Inc, Chicago, IL). Pearson's Chi Square test was used for testing the categorical data of genotype association with breast cancer and estimation of Hardy Weinberg Equilibrium (WHE). Allele frequencies were calculated by gene counting method. The relation of I/D alleles with the presence of breast cancer was also tested considering both recessive effect of deletion allele (DD vs. DI+II) and a dominant effect of same allele (DD+DI vs. II) [39] the strength of given gene cancer association was measured by the odd ratio (OR) and corresponding 95% confidence interval (95% CI) and two sided p value. P value < 0.05 was considered statistically significant.

3. Results:

A total of 97 subjects (36 cases and 61 controls) were included in this case control study. All of samples were subjected to analyses for I/D polymorphisms of the ACE gene and agarose gel electrophoresis illustrated two bands insertion 490 bp and deletion 190 bp were visible (Figure 1). Subjects were classified

according to the presence or absence of a 287 base pairs insertion in intron 16 of ACE gene as II, ID, DD. When DD sample amplified using the I specific primer giving 335 bp fragment it was recorded as ID variant (Figure 2).

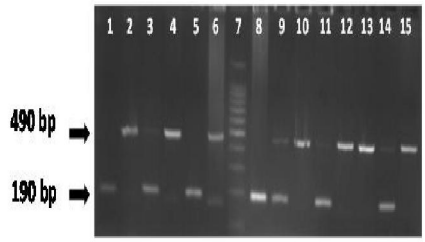

Figure 1: electrophoresis of ACE I/D : lanes1,3,5,8,11 are DD homozygote with single band at 190, lanes 2,4,10,12,13 are II homozygote with one band 490, Lanes 6,9,14 are ID heterozygotes with two bands, and lane 7 is 100 bp marker.

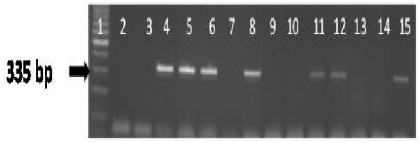

Figure 2: electrophoresis using insert specific primers: lanes 4,5,6,8,11,12,15 show single bands of the insertion fragment of 335 bp so considered ID heterozygote, while lanes 2,3,7,9,10, 13, 14 no bands indicating DD homozygote, lane 1 shows 100 bp marker.

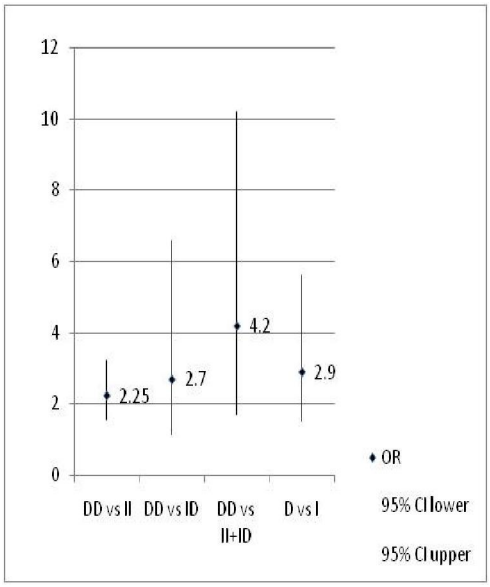

Figure 3: Risk of breast cancer in comparing different variants of I/D polymorphism of ACE gene

The frequency of I/D genotype of ACE gene were in hardy Weinberg equilibrium (χ^2 = 1.78, P>0.05).

As shown in Table (1) the difference in distribution of I/D genotype in patient and control group was statistically significant, as 20 (55.6%) of patients had DD, 16(44.4%) had ID and no patients (0%) had II genotype. DD was present in 16 (26.2%), ID in 35 (57.4%) ` II in 10(16.4%) of healthy subjects (χ^2 = 11.8, P=0.003),

As shown in Table (2) and figure (3) There was significant association of D allele and breast cancer incidence (77.7% of cases, and 54.9% of control) when compared with I allele (22.2% of cases, and 45.1% of control), $\chi 2$ = 10.1, P=0.001, OR=2.9, 95%CI= 1.5-5.6. The risk was increased in homozygous DD genotype carriers in comparison to other genotypes (OR=4.2, 95% CI=1.7-10.2, χ^2 = 10.6, P=0.001). No significant difference was found between genotype and allele frequency in patients or control group.

Pathological parameters of malignant patients are shown in table (3)

The frequency of DD genotype was higher in patients with high histological grade of tumor, DD was 66.7%, ID was 33.3% (OR=2, 95% CI=0.47-8.46) and in patients with advanced stages DD was 60%, ID was 40% (OR=2.5, 95% CI 0.65-9.6). Less risk was noticed in large tumor size DD was 53.3%, ID was 46.7% (OR=1.3, 95% CI=0.29-5.6). But no association was noticed between DD genotype and lymph node involvement Table (4).

4. Discussion:

Breast cancer is widely spread tumor, it accounts for 18% of all female malignancies **[Perera and Gui 2003; Prichard *et al.*, 2003].** Breast cancer is the second leading cause of cancer death in women, exceeded only by lung cancer. The chance that breast cancer will be responsible for a woman's death is about 1 in 35 (about 3%) **[American cancer society, 2011].** There are several risk Factors predisposing to this disease including genetic factor **[Singletary SE 2003].** Which are estimated to predispose for 15-25% of the cases **[Mitrunen and Hirvonen 2003].** Several studies have shown that angiotensin II acts as growth factor for normal and breast cancer cells through phospholipase C activation **[Greco *et al.*, 2002; and 2003, De Paepe *et al.*, 2002].** Literatures proved association of RAS inhibitors with decreased risk of malignancy in individuals with DD genotype which is associated with high level of ACE **[Van der Knaap *et al.*, 2008].** Our results indicated significant increase in risk of breast cancer incidence in D allele and homozygous DD genotype carriers than I allele and II genotype OR= 2.9 (95% CI 1.5-5.6), 4.2 (95% CI 1.7-10.2) respectively.

Table (1): Distribution of I/D alleles and genotypes in both malignant and control groups.

	Control group % (n=61)	Malignant group % (n=36)	χ^2	P
II genotype	16.4%(10)	0%(0)	11.8	0.003*
ID genotype	57.4%(35)	44.4%(16)		
DD genotype	26.2%(16)	55.6%(20)		
D allele	54.9% (67/122)	77.7%(56/72)	10.14	0.001*
I allele	45.1% (55/122)	22.2% (16/72)		

*p<0.05 is significant

Table (2): Distribution of ACE I/D genotype and allele frequency in relation to breast cancer risk

		Control % (n)	Malignant	OR	95% CI	χ^2	P
DD vs. II	DD	61.5% (16/26)	100% (20/20)	2.25	1.56- 3.24	9.8	0.002*
	II	38.5% (10/26)	0% (0/20)				
DD vs. ID	DD	31.4% (16/51)	55.5% (20/36)	2.7	1.13-6.6	5.1	0.03*
	ID	68.6% (35/51)	44.4%(16/36)				
DD vs. II+ID	DD	23% (14/61)	55.6%(20/36)	4.2	1.7-10.2	10.6	0.001*
	II+ID	77% (47/61)	44.4%(16/36)				
II vs. DD+ID	II	19.7% (12/61)	0%(0/36)	0.58	0.48-0.69	8.1	0.002*
	DD+ID	80.3% (49/61)	100%(36/36)				
D allele vs. I allele	D	54.9% (67/122)	77.7% (56/72)	2.9	1.5-5.6	10.1	0.001*
	I	45.1% (55/122)	22.2% (16/72)				
ID vs. II	ID	77.8% (10/45)	100%(16/16)	1.46	1.2-1.8	4.3	0.04*
	II	22.2% (35/45)	0%(0/16)				

*Statistically Significant, OR= Odd ratio, 95% CI= 95% confidence interval

Table (3): Pathological parameters of malignant patients

Malignant samples		%(frequency)
Stage	IIA	22.2%(8)
	IIB	27.8%(10)
	IIIA	27.8%(10)
	IIIC	5.6%(2)
	IV	16.7%(6)
Grade	1	11.1%(4)
	2	55.6%(20)
	3	33.3%(12)
Distant metastasis	negative	83.3%(30)
	positive	16.7%(6)
Lymph nodes	negative	16.7%(6)
	positive	83.3%(30)
Tumor size	≤2cm	72.2%(26)
	>2cm	27.8%(10)
Pathology	Invasive duct carcinoma	58.3%(21)
	Mixed ductal and lobular.	19.4%(7)
	Paget's disease	11.1%(4)
	Poorly differentiated tumor	11.1%(4)

Table (4): Association of ACE I /D genotypes pathological parameters of breast cancer patients

		ID	DD	Total	OR	CI	χ^2	P
Stage	Early(IIA,IIB)	55.6%(10)	44.4%(8)	18	2.5	0.65-9.6	8.3	> 0.05
	Late(IIIA,B,IV)	33.3%(6)	66.7%(12)	18				
Distant metastasis	negative	46.7%(14)	53.3%(16)	30	1.75	0.27-11.04	0.36	> 0.05
	positive	33.3%(2)	66.7%(4)	6				
Grade	Low	50%(12/24)	50%(12/24)	24	2	0.47-8.46	0.9	> 0.05
	High	33.3%(4/12)	66.7%(8/12)	12				
Tumor size	≤2 cm	46.2%(12)	53.8%(14)	26	1.3	0.29-5.6	0.11	> 0.05
	>2 cm	40%(4)	60%(6)	10				
Lymph nodes	negative	33.3%(2)	66.7%(4)	6	0.57	0.09-0.36	0.36	> 0.05
	positive	46.7%(14)	53.3%(16)	30				

*Statistically Significant, OR= Odd ratio, 95% CI= 95% confidence interval

This was in agreement with previous studies which has reported association of DD genotype with increase breast cancer risk OR= 1.86 , 80% increase risk in DD genotype **[Vairaktaris *et al*., 2007, Toma *et al*.,2009**], while the low activity II genotype exhibited lower breast cancer risk OR was 0.46 **[Gonzalez-Zuloeta Ladd *et al*., 2005].** A study by **Alves correa *et al*., 2009** found that ID genotype carriers (20% in cases and 37% in control) were 3.1 times less likely to develop breast cancer than those with other genotypes DD (60% in cases and 46% in control) and II (20% in cases and 17% in control) and that ACE I/D polymorphism is a possible target for developing genetic marker of breast cancer**,** association of ACE I/D polymorphism with other malignancies was designated in studies on prostate cancer there was high risk for DD genotype (OR 31.6, 95%CI 0.09-1.27) **[Sierra Díaz *et al*., 2009],** and there risk for early gastric carcinoma development was significantly lower in patients with ACE II and ID than those with DD genotype (ORs 0.2, and 0.55) respectively **[Ebert *et al*., 2005]** [27]**.** In lung cancer patients allelic frequencies and genotype distribution of the ACE I/D polymorphism in the patient group were significantly different from control subjects (ACE II genotype 29.6 vs. 17.6%, P = 0.011; ACE I allele 49.6 vs. 39.4%, P =0.009), suggesting that the ACE I/D polymorphism could be a risk factor for patients with lung cancer **[Nacak *et al*., 2010].** A study on patients with gall bladder cancer reported increase risk in DD carriers which is significant among women OR = 1.63; p = 0.039 **[Srivastava *et al*., 2010].**

On contrary to our results a multiethnic study by **Haiman *et al*., 2003** reported that women with II genotype had marginally significant breast cancer risk OR=1.3 995% CI 1.05-1.61)**,** a further study on multiethnic group found no correlation between ACE polymorphism and breast cancer incidence but this finding was not observed in all studied ethnic groups **[Van der Knaap *et al*., 2008].** I allele and II genotype associated with increased risk of oral oncogenesis OR 3.17, 95% C.I. 1.32-7.61 **[Vairaktaris *et al*., 2007],** and previous studies showed no relation of ACE gene polymorphism to lung and colorectal cancer **[Toma *et al*., 2009, Cheon *et al*., 2000].**

The controversy in results may be a result of difference in tumor tissues, and /or ethnic groups, this view was supported by meta-analysis done by **Loh *et al*., 2009** between studies on gastric cancer, who found significant difference odd ratios of ACE I/D between different racial groups**.**

In the present study there was association between DD genotype and incidence of high grade OR= 2 (95%CI 0.47-8.46), advanced stage OR= 2.5 (95%CI 0.65-9.6), and less risk for large tumor size OR= 1.3 (95%CI 0.29-5.6) but there was no association noticed with lymph node involvement, these findings were in accordance with other studies who linked breast cancer progression with gene polymorphism as in breast cancer patients, at which DD genotype was associated with increased HER-2 expression (P = 0.020; OR, 4.58; 95% CI, 1.26-16.60) as compared to II and ID genotypes **[Namazi *et al*., 2010].** Another study found association with large tumor size **[Yaren *et al*., 2006].** This may be a consequence of angiogenic effect of angiotensin II which increase tumor cell proliferation through increased VEGF gene expression **[Yoshiji *et al*., 2001].**Thus influence the local tumor growth in breast cancer. In other malignancies ACE polymorphism was association with prognostic parameters, in gastric cancer patients the number of lymph node metastasis and clinical stage correlated with DD genotype there was no correlation between tumor type, location, local tumor growth, distant metastasis, and I/D polymorphism **[Röcken *et al*., 2005].** In patients with prostatic cancer presentation with more advanced tumors were observed in DD carriers **[Nacak *et al*., 2010].** And **Rochen *et al*., 2007** found that ACE polymorphism was associated

with gender specific difference in primary tumor size and patient survival**.**

On the light of the present study we agreed with studies that prove the role of high activity DD genotype in increasing the risk of breast cancer and potential effect on its progress, so we recorded using drugs that decrease level of angiotensin II in hypertension as preventive and therapeutic drugs for breast cancer.

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References:

Ali Eldin N. (2010). Cancer statistics 2002 – 2007.Preliminary report**.** Department of Biostatistics and Cancer Epidemiology National Cancer Institute. **[http://www.nci.cu.edu.eg/lectures/Cancer_statistics 2002-2007.pdf].**

Alves Corrêa S.A.; Ribeiro de Noronha S.M.; Nogueira-de-Souza N.C.; Valleta de Carvalho C.; Massad Costa A.M.; Juvenal Linhares J.; Vieira Gomes M.T.; and Guerreiro da Silva I.D. (2009). 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. (1):51-8.

American cancer society (2011). Key statistics about breast cancer. http://www.cancer.org/Cancer/BreastCancer/DetailedGuide/breast-cancer-key-statistics.

Cheon K.T.; Choi K.H.; Lee H.B.; Park S.K.; Rhee Y.K.; and Lee Y.C. (2000). Gene polymorphisms of endothelial nitric oxide synthase and angiotensin-converting enzyme in patients with lung cancer. Lung. ; 178:351–360.

Copeland G.; Lake A.; Firth R.; Lehnherr M.; Bayakly R.;Wu X.C.; Stroup A.;Russell C.; Schymura M.; Hofferkamp J.; and Kohler B. (2009). Cancer in North America: 2002-2006. Volume One: Combined Cancer Incidence for the United States and Canada. Springfi eld, IL: North American Association of Central Cancer Registries, Inc. June 2009. [www.naaccr.org/ Data and Publications/PreviousCINAPubs.aspx.**].**

De Paepe B.; Verstraeten V.M.; De Potter C.R.; and Bullock G.R. (2002). Increased angiotensin II type-2 receptor density in hyperplasia, DCIS and invasive carcinoma of the breast is paralleled with increased iNOS expression. Histochem Cell Biol; 117:13–9.

Ebert M.P.; Lendeckel U.; Westphal S.; Dierkes J.; Glas J.; Folwaczny C.; Roessner A.; Stolte M.; and Röcken C. (2005). The angiotensin I-converting enzyme gene insertion/deletion polymorphism is linked to early gastric cancer. Cancer Epidemiol Biomarkers Prev; 14:2987–9.

Emad A.; Rakha Maysa E.; Andrew H.S.; Christopher W. E.; Matthew J. G.; Roger W. B.; and Ian O. E. (2008). Prognostic Significance of Nottingham Histologic Grade in Invasive Breast Carcinoma. Journal of Clinical Oncology (26):19.

Eva Singletary S.; and James Connolly L. (2006). Breast Cancer Staging: Working With the Sixth Edition of the AJCC Cancer Staging Manual. American Cancer Society, CA Cancer J Clin 2006; 56:37-47.

Folkman J.; and Shing Y. (1992). Angiogenesis. J Biol Chem; 267:10931–4.

Gonzalez-Zuloeta Ladd A.M..; Vasquez A.A.; Sayed-Tabatabaei F.A.; Coebergh J.W.; Hofman A.; Njajou O.; Stricker B.; van Duijn C. (2005). Angiotensin-converting enzyme gene insertion/deletion polymorphism and breast cancer risk. Cancer Epidemiol Biomarkers Prev ;14:2143–6

Greco S.; Muscella A.; Elia M.G.; Salvatore P.; Storelli C.; and Marsigliante S. (2002). Activation of angiotensin II type I receptor promotes protein kinase C translocation and cell proliferation in human cultured breast epithelial cells. J Endocrinol; 174:205–14

Greco S.; Muscella A.; Elia M.G.; Salvatore P.; Storelli C.; Mazzotta A.; Manca C.;and Marsigliante S.(2003). Angiotensin II activates extracellular signal regulated kinases via protein kinase C and epidermal growth factor receptor in breast cancer cells. J Cell Physiol; 196:370–7.

Haiman C.A.; Henderson S.O.; Bretsky P.; Kolonel L.N.; Henderson E. (2003). Genetic variation in angiotensin I-converting enzyme (ACE) and breast cancer risk: the multiethnic cohort. Cancer Res.; 63:6984–6987.

Hii, S.I.; Nicol, D.L.; Gotley, D.C.; Thompson, L.C.; Green, M.K.; and Jonsson, J.R. (1998). Captopril inhibits tumor growth in xenograft model of human renal cell carcinoma. Br. J. Cancer, 77: 880-883.

Hubert C.; Houot A.M.; Corvol P.; and Soubrier F. (1991). Structure of the angiotensin I-converting enzyme gene: two alternate promoters correspond to evolutionary steps of a duplicated gene. J. Biol. Chem., 266: 15377-15383.

Josef S; David WR; Nina I; Kaaren A.J. (2002). Molecular Cloning: Rapid Isloation of Mammalian DNA. Cold Spring Harbour Laboratory Press. New

York P628-630.

Koh W.P.; Yuan J.M.; Sun C.L.; Van den Berg D.; Seow A.; Lee H.P.; and Yu M.C. (2003). Angiotensin I-converting enzyme (ACE) gene polymorphism and breast cancer risk among Chinese women in Singapore. Cancer Res; 63:573–8.

Koh W.P.; Yuan J.M.; van den Berg D.; Lee H.P.; and Yu M.C. (2005). Polymorphism in angiotensin II type 1 receptor and angiotensin I-converting enzyme genes and breast cancer risk among Chinese women in Singapore. Carcinogenesis, 26: 459-464.

Loh M.; Koh K.X.; Yeo B.H.; Song C.M.; Chia K.S.; Zhu F.; Yeoh K.G, Hill J.; Iacopetta B.; and Soong R. (2009). Meta-analysis of genetic polymorphisms and gastric cancer risk: variability in associations according to race. Eur J Cancer. 45(14):2562-8.

Mayer N.J.; Forsyth A.; Kantachuvesiri S.; Mullins J.J.; Fleming S. (2002). Association of the D allele of the angiotensin I converting enzyme polymorphism with malignant vascular injury. Mol Pathol. Feb; 55(1):29-33.

Medeiros R.; Vasconcelos A.; Costa S.; Pinto D.; Lobo F.; Morais A.; Oliveira J.; Lopes C. (2004). Linkage of angiotensin I-converting enzyme gene insertion/deletion polymorphism to the progression of human prostate cancer. J Pathol; 202:330–5.

Mitrunen K.; Hirvonen A. (2003). Molecular epidemiology of sporadic breast cancerThe role of polymorphic genes involved in oestrogen biosynthesis and metabolism. Mutat Res; 544:9–41.

Nacak M.; Nacak I.; Sanli M.; Ozkur M.; Pektaş M.; Aynacioğlu A.S. (2010). Association of angiotensin converting enzyme gene insertion/deletion polymorphism with lung cancer in Turkey. Cancer Genet. Cytogenet.; 198(1):22-6.

Namazi S.; Monabati A.; Ardeshir-Rouhani-Fard S.; Azarpira N. (2010). 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.

Perera N.M.; Gui G.P. (2003). Multi-ethnic differences in breast cancer: current concepts and future directions. Int J Cancer; (2003)106:463–7.

Prichard RS, Hill AD, Dijkstra B, McDermott EW, O'Higgins NJ. (2003). The prevention of breast cancer. Br J Surg; 90 (97):772–83.

Röcken C.; Lendelkel U.; Dierkes J.; Westphal S.; McGrath S.C.; Peters B.; Kruger S.; Malfertheiner P.; Roessner A.; and Ebert M.P. (2005). The number of the lymph node metastasis in gastric cancer correlates with the Angiotensin I-converting enzyme gene Insertion/Deletion polymorphism. Clin. Cancer Res., 11: 2526-2530.

Röcken, C.; Neumann, K.; Carl-McGrath S.; Lage H.; Ebert M.; Dierkes J.; Jacobi C.; Kalmuk S.; Neuhaus P.; and Neumann U. (2007). The Gene Polymorphism of the Angiotensin I-Converting Enzyme Correlates with Tumor Size and Patient Survival in Colorectal Cancer Patients. Neoplasia. 9(9): 716–722.

Rueckschloss, U.; Quinn,M.T.; Holtz,J.; and Morawietz,H. (2002). Dose-dependent regulation of NAD (P) H oxidase expression by angiotensin II in human endothelial cells: protective effect of angiotensin II type 1 receptor blockade in patients with coronary artery disease. Arterioscler. Thromb. Vasc. Biol., 22:1845–1851.

Saslow D.; Boetes C.; Burke W.; Harms S.; Leach M.O.; Lehman C.D.; Morris E.; Pisano E.; Schnall M.; Sener S.; and Smith R.A. (2007). Warner E, Yaffe M, Andrews KS, and Russell CA. American Cancer Society guidelines for breast screening with MRI as an adjunct to mammography. CA Cancer J Clin. ; 57: 75-89.

Sayed-Tabatabaei F.A.;Schut A.F.;Hofman A.; Bertoli-Avella A.M.; Vergeer J.; Witteman J.C.; Van Duijn C.M. (2004). A study of gene–environment interaction on the gene for angiotensin converting enzyme: a combined functional and population based approach. J Med Genet. ; 41: 99–103.

Shih, H.A.; Couch, F.J.; Nathanson, K.L.; Blackwood, M.A.; Rebbeck, T.R.; Armstrong, K.A.; Calzone, K.; Stopfer, J.; Seal, S.; and Stratton M.R. (2002). BRCA1 and BRCA2 mutation frequency in women evaluated in a breast cancer risk evaluation clinic. J. Clin. Oncol., 20: 994-999.

Siani A.; Russo P.; Paolo Cappuccio F.; Iacone R.; Venezia A.; Russo O.; Barba G.; Iacoviello L.; and Strazzullo P. (2004). Combination of renin-angiotensin system polymorphisms is associated with altered renal sodium handling and hypertension. Hypertension. 43:598–602.

Sierra Díaz E.; Sánchez Corona J.; Rosales Gómez R.C.; Gutierrez Rubio S.A.; Vázquez Camacho J.G.; Solano Moreno H.; and Morán Moguel M.C. (2009). Angiotensin-converting enzyme insertion/deletion and angiotensin type 1 receptor A1166C polymorphisms as genetic risk factors in benign prostatic hyperplasia and prostate cancer. Renin Angiotensin Aldosterone Syst. (4):241-6.

Singletary S.E. (2003). Rating the risk factors for breast cancer. Ann Surg; 237:474–82.

Srivastava K.; Srivastava A.; Mittal B. (2010). Angiotensin I-converting enzyme insertion/deletion polymorphism and increased risk of gall bladder

cancer in women. DNA Cell Biol. (8):417-22.

Toma M.; Cimponeriu D.; Apostol P.; Stavarachi M.; Cojocaru M.; Belusică L.; Crăciun A.M.; Radu I; and Gavrilă L. (2009). Lack of association between ACE ID polymorphism and colorectal cancer in Romanian patients. Chirurgia (Bucur). 104(5):553-6.

Vairaktaris E.; Yapijakis C.; Tsigris C.; Vassiliou S.; Derka S.; Nkenke E.; Spyridonidou S.; Vylliotis A.; Vorris E.; Ragos V.; Neukam F.W.; and Patsouris E. (2007). Association of angiotensin-converting enzyme gene insertion/deletion polymorphism with increased risk for oral cancer. Acta Oncol. (8):1097-102.

Van der Knaap R.; Siemes C.; Coebergh J.W.; Van Duijn C.M.; Hofman A.; Stricker B. H. (2008). Renin-angiotensin system inhibitors, angiotensin I-converting enzyme gene insertion/deletion polymorphism, and cancer: the Rotterdam Study. Cancer; 112(4):748-57.

Walther T.; Menrad A.; Orzechowski H.D.; Siemeister G.; Paul M.; and Schirner M.; (2003). Differential regulation of in vivo angiogenesis by angiotensin II receptors. FASEB J; 17:2061–7.

Yaren A.; Turgut S.; Kursunluoglu R.; Oztop I.; Turgut G.; Kelten C.; and Erdem E. (2006). Association between the polymorphism of the angiotensin-converting enzyme gene and tumor size of breast cancer in premenopausal patients.Tohoku J Exp Med. 210 (2):109-16.

Yoshiji H.; Kuriyama S.; Kawata M.; Yoshii J.; Ikenaka Y.; Noguchi R.; Nakatani T.; Tsujinoue H.; and Fukui H. (2001). The angiotensin converting enzyme inhibitor perindopril suppresses tumor growth and angiogenesis: possible role of vascular endothelial growth factor. Clin. Cancer Res., 7: 1073-1078.

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