Original Article

ACE gene polymorphism in breast cancer patients of ethnic Kashmiri population

Abstract

Background: The mitogenic and angiogenic effect of angiotensin II has been shown in breast cancer. Angiotensin II is converted from its inactive form to active form by the angiotensin I converting enzyme (ACE). **Materials and Methods:** To evaluate the effect of ACE gene in breast cancer patients and its effect on healthy control subjects, we studied 130 breast cancer patients and 220 healthy controls. **Results:** During our study, we found, the ACE genotype distribution in breast cancer patients were as follows: 62 (47.6%) had Deletion (DD), 43 (33.07%) had ID, and 25 (19.23%) had II (Insertion) genotypes, whereas in controls, 96 (43.63%) had DD, 107 (48.63%) had ID, and 25 (7.7%) had II genotypes. **Conclusion:** We conclude that our results implicate that ACE level/activity has been suggested to be protective against breast cancer, and therefore renin-angiotensin system may serve as a curative target for breast cancer detection, treatment, and prevention. **Impact:** Our study is the first report from India on Kashmiri population, suggesting that ACE activity can be a protective tool against breast cancer.

Access this article online

Website
http://www.cysonline.org

DOI
10.4103/2229-5186.76986

Quick Response Code

Key words:
ACE, breast cancer, polymorphism

Introduction

Breast cancer, is the third most common malignancy in the world,[1] with more than 1 million women diagnosed with breast cancer each year.[2] The disease has been associated with a variety of risk factors,[3] genetic and epigenetic changes.[4] But its molecular pathogenesis remains unresolved. Environmental carcinogens have been shown to damage DNA at active fragile sites by disrupting surveillance, which has been shown to be tumorigenic.[5,6] Development of human breast cancers is a multistep process, arising from genetic alterations that drive the transformation of normal mammary epithelial cells into highly malignant derivatives.[7]

Angiotensin I-converting enzyme is an exopeptidase circulating enzyme that participates in the body's renin-angiotensin system (RAS), which mediates extracellular volume (that of the blood plasma, lymph, and interstitial fluid) and arterial vasoconstriction. It is secreted by pulmonary and renal endothelial cells and catalyses the conversion of decapeptide angiotensin I to octapeptide angiotensin II.[8] Angiotensin II is an aldosterone-stimulating peptide with a direct, potent vasopressive effect on the peripheral vasculature. It plays a pivotal role in electrolyte and circulatory homeostasis. It is converted from its precursor, angiotensin I, by the catalytic action of the dipeptidyl carboxypeptidase known as ACE,[9] which is present as a membrane-bound enzyme on the surface of a variety of cell types as well as in a secreted form.

The ACE polymorphism was first identified in 1990 by Rigat and coworkers,[10] the gene encoding ACE is located on the long arm of chromosome 17 (17q23). The gene is 21 kb long and comprises 26 exons and 25 introns. The principle of ACE gene polymorphism can be detected by the presence or absence of a 287-bp *Alu* insertion/

Nidda Syeed[1,4], Safiya Abdullah[1], A. Syed Sameer[1,2], Syed Akhtar Husain[4], Saniya Nisar[3], Afshana Rasool[3], Mushtaq A. Siddiqi[4]

Departments of [1]Immunology & Molecular Medicine and [2]Clinical Biochemistry, Sher-I-Kashmir Institute of Medical Sciences, Soura, Srinagar, Kashmir - 190 011, [3]Department of Clinical Biochemistry, University of Kashmir, Hazratbal - 190 006, [4]Department of Biosciences, Jamia Millia Islamia, New Delhi - 110 011, India.

Address for correspondence:
Prof. Mushtaq A. Siddiqi
Department of Immunology and Molecular Medicine, Sher-I-Kashmir Institute of Medical Sciences, Soura, India.
E-mail: mousvi786@gmail.com

How to cite this article: Syeed N, Abdullah S, Sameer AS, Husain SA, Nisar S, Rasool A, *et al.* ACE gene polymorphism in breast cancer patients of ethnic Kashmiri population. Chron Young Sci 2010, 1:40-5

deletion (I/D) polymorphism in intron 16 on chromosome 17. A study by Talamini and Peeters[11,12] has shown that hypertension during pregnancy and after menopause has been positively associated with breast cancer risk in a small number of studies. Furthermore, Muscella and Fujita have reported a direct involvement of angiotensin II in breast cell proliferation,[13] angiogenesis, and tumour metastasis[14] that are considered to be a significant means associated in the development and progression of breast cancer.

According to Koh *et al*,[15] the relationship between the ACE activity and Angiotensin II with breast cancer, hypothesised that women carrying the low activity (A and I) alleles of the ACE A-240T and I/D polymorphisms would have lower ACE levels, and furthermore, a decrease in the synthesis of Ang II, would be less prone in developing breast cancer. In addition, Haiman *et al*,[16] also studied I/D and A-240T polymorphisms in the ACE gene in relation to the risk of developing breast cancer. In addition to this, Haiman *et al*, also found that women with the II genotype had a significant increase in breast cancer. They suggested that the ACE (I/D) polymorphism is not likely to be a strong predictor of breast cancer risk.

Looking at the present literature, we devised our present study to evaluate the I/D polymorphisms of the ACE gene in breast cancer patients and the control group of our population.

Materials and Methods

Patients and tumour tissue procurement

All breast cancer patients included in the study were both male and female, with the histopathological diagnosis of the breast cancer. Patient participation was obtained through informed consent and after approval from the Ethics Committee of Sher-I-Kashmir Institute of Medical Sciences.

A cohort of 130 breast cancer tissue samples were collected consisting of tumour tissues and adjacent normal tissue. Only the tissue samples confirmed by histopathological studies to be cancerous were included in the study.

Controls

Patients attending the Department of General Medicine at Sher-I-Kashmir Institute of Medical Sciences (SKIMS) for general checkup were screened. A total of 260 patients visited the SKIMS, and of 260 patients, only 220 agreed to participate in the present study; thus, written informed consent was obtained from all patients for their participation.

DNA isolation

Genomic DNA was extracted from tissue samples and peripheral blood samples of breast cancer patients by using DNA Extraction Kit (Qiagen, USA). The quality of the resulting genomic DNA was stringently assessed by low-percentage agarose gel electrophoresis.

ACE gene polymorphism

Total genomic DNA was extracted from whole blood, as described earlier. The insertion-deletion polymorphism of 287 base pairs (bp) in intron 16 of the ACE gene was identified by conventional polymerase chain reaction (PCR) by using two primers flanking the site of insertion [Table 1].[16-18] Fragments of about 190 bp (*D* allele) and 490 bp (*I* allele) were separated on 2% agarose gel. All samples that seemed homozygous for the *D* allele were subjected to a second PCR amplification, with an insertion-specific primer to check for misclassification resulting from a potential preferential amplification of the smaller *D* allele.[17,18] About 4-5% of individuals with the ID genotype were initially misclassified as having the DD genotype, but this error was corrected before statistical analysis [Figure 1].

Statistical analysis

Observed frequencies of genotypes in cancer patients were compared to controls using Chi-square or Fisher exact tests when expected frequencies were small. The Chi-square test was used to verify whether genotype distributions were in Hardy–Weinberg equilibrium. Statistical significance was set at $P<0.05$. Statistical analyses were performed with PASW version 18 Software.

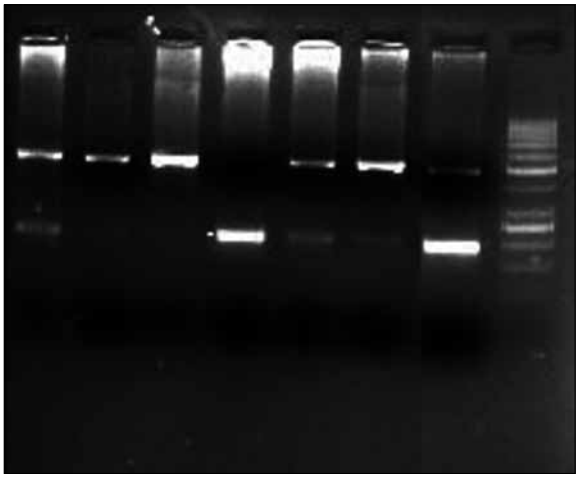

Figure 1: Representative gel picture of ACE DI polymorphism by differential amplification of intron 16 of the ACE gene

Table 1: Primer sequence used to amplify ACE gene

Primer sequence	Product size	Tm (oC)
F: 5′-TGGAGACCACTCCCATCCTTTCT-3′	490 bp for II	
R: 5′- GATGTGGCCATCACATTCGTCAGAT-3′	190 bp for DD	58
F: 5′-TGGGACCACAGCGCCCGCCACTAC-3′		
R: 5′-TCGCCAGCCCTCCCATGCCCATAA-3′	335 bp	67

Results

For the present study, we randomly selected 130 breast cancer patients and 220 control subjects. The mean age of the cases and controls was similar within our ethnic group, ie, 52 years for cases and 52 years for controls. The frequency of ACE genotype with respect to breast cancer patients and control subjects is shown in Tables 2 and 3. The ACE genotype distribution in breast cancer patients were as follows: 62 (47.6%) had DD, 43 (33.07%) had ID, and 25 (19.23%) had II, while in controls, 96 (43.63%) had DD, 107 (48.63%) had ID, and 25 (7.7%) had II genotypes.

The correlation of ACE gene polymorphic status and clinicopathologic characteristics was carefully analysed. The DD genotype was found to be significantly related to smoking status (Ever Smokers), menopausal status (Pre), and tumour stage (III+IV) [Table 4].

In this study, we observed that genotype frequencies in cases and controls were in Hardy–Weinberg equilibrium. The genotype frequencies of ACE gene in cases and controls were observed, and it was found that II genotype is significantly associated with the breast cancer cases (P=0.001). No significant gender- or age-related differences were observed between the groups (P>0.05) [Table 2].

Discussion

Breast cancer is one of the most common malignancies in women. It continues to be a major burden and cause of death among women worldwide. Molecular oncology is a promising field that may contribute considerably to diagnosis of breast cancer and its metastases, addressing major problems with early detection, accurate staging, and monitoring of breast cancer patients. The development of human breast cancer is a multistep process, arising from genetic alterations that drive the transformation of

Table 2: Genotype frequencies of ACE gene in cases and controls, and their associations with the risk of breast cancer patients of Kashmir valley

ACE Genotype	Cases (n=130)	Controls (n=220)	χ^2; P value
DD	62 (47.6%)	96 (43.63%)	
ID	43 (33.07%)	107 (48.63%)	8.43; 0.014
II	25 (19.23%)	25 (7.7%)	

Table 3: Genotype frequencies of ACE gene in cases and controls, and their associations with the risk of breast cancer patients of Kashmir valley

ACE Genotype	Cases (n=130)	Controls (n=220)	OR (95% CI); P value
DD	62 (47.6%)	96 (43.63%)	1 (Ref)
ID	43 (33.07%)	107 (48.63%)	0.62 (0.39-1.0)
II	25 (19.23%)	25 (7.7%)	1.54 (0.82-2.93)
ID+II	68 (52.3%)	131 (56.33%)	0.80 (0.52-1.23)

Table 4: Association between ACE genotypes and clinicopathologic characteristics

Variables	Cases (n=130)				
	Total N= 30 (%)	DD 62 (47.6%)	ID 43 (33.07%)	II 25(19.23%)	χ2; P Value
Dwelling					
Rural	45 (34.6)	17 (27.41)	15 (34.88)	13 (52.0)	4.76
Urban	85 (65.4)	45 (72.58)	28 (65.11)	12 (48.0)	0.09
Smoking status					
Never	93 (71.5)	38 (61.29)	36 (83.72)	19 (76.0)	6.58
Ever	37 (28.5)	24 (38.70)	7 (16.27)	6 (24.0)	0.03
Menopausal status					
Pre	36 (27.7)	11 (17.74)	13 (30.23)	12 (48.0)	8.35
Post	94 (72.3)	51 (82.25)	30 (69.76)	13 (52.0)	0.01
Nodal status					
Involved	34 (26.2)	13 (20.96)	11 (25.58)	10 (40.0)	3.35
Not involved	96 (73.8)	49 (79.03)	32 (74.41)	15 (60.0)	0.18
Breast involved					
R	35 (26.9)	12 (19.35)	12 (27.90)	11 (44.0)	5.53
L	95 (73.1)	50 (80.64)	31 (72.09)	14 (56.0)	0.06
Tumor stage					
II (a+b)	72 (55.4)	27 (43.54)	26 (60.46)	19 (76.0)	8.26
III (a+b)+IV	58 (44.6)	35 (56.45)	17 (39.53)	6 (24.0)	0.01
Histopathological tumour grade					
PD	25 (96.0)	17 (27.41)	14 (32.55)	7 (28.0)	0.47
MD+WD	58 + 47 (24.1)	45 (72.58)	29 (67.44)	18 (72.0)	0.79

normal mammary epithelial cells into highly malignant derivatives.

Angiotensin II is a potent angiogenic factor reported to participate in the development and promotion of tumorigenesis.[19-21] Angiotensin II upregulates NADPH oxidase in endothelial cells in a dose-dependent manner,[22,23] and the latter is an important source in the production of Reactive Oxygen Species (ROS). ROS participates in vascular endothelial growth factor (VEGF) signalling and plays a potential role in VEGF-induced angiogenesis *in vitro* as well as *in vivo*.[24] VEGF has also been identified to play a major role in promoting neovascularisation in human breast cancer.[25] A recent study[26] has reported that the DD genotype might be an adjunct with poor prognostic factors and influence the tumour course. The frequency of the ID genotype in this study was higher in breast cancer patients than that in the control group.

The main aim of our study was to predict the predisposition towards development of breast cancer due to different ACE levels. The present study was carried out in 130 breast cancer samples and 220 control samples.

All patients and controls were collected from the Kashmir valley only, the strength of the study being the place where it is studied. As reported earlier, breast cancer is a heterogeneous disease, and several risk factors are responsible in its development.[27,28] Kashmir valley is located in the northern most part of India. The Kashmir valley lies between Himalayas and the Pir Panjal Range, about 5,000 ft above the sea level. The genetic makeup of the Kashmiri population is preserved due to consanguineous marriage that has led to the preservation of the genetics of this population. The people of Kashmir constitute a different set of dietary habits that have high percentage of nitroso compounds, amines, and nitrates present in local food material.[29-31] The local food material consist of dried and smoked fish and meat, dried and pickled vegetables, red chilli, Hakh (a leafy vegetable of Brassica family), hot noon chai (salted tea), and Hukka (water pipe) smoke.

The ACE gene frequency in our cases is as follows: 62 (47.6%) had DD, 43 (33.07%) had ID and 25 (19.23%) had II, whereas 96 (43.63%) had DD, 107 (48.63%) had ID, and 25 (7.7%) had II genotypes, in healthy controls.

Our results were consistent with the findings of an earlier study,[15] in which breast cancer patients with ACE genotype having II genotype for *D/I* gene polymorphisms were at lower risk for breast cancer when compared to its counterparts with the ACE genotypes DD. The I/D in the ACE gene might not play a direct role in regulating the ACE transcription; instead, it is in linkage disequilibrium with the regulatory elements of ACE gene. Our results were consistent with the results of earlier studies that were done in multi-ethnic cohort[32] for the positive association between the I/I ACE genotype and breast cancer risk. We found a significant association between the I/D genotype and the controls, showing that these carry the lowest risk for breast cancer, as has been seen earlier in the Chinese population.[15]

We found a significant association of the DD genotype in cases with smoking status, showing that smoking might be a risk factor for breast cancer. Further, we found a significant association between the DD genotype and the premenopausal status, with the reason that estradiol was a more potent regulator of free VEGF levels, which is supposedly involved in mammary carcinogenesis, and hence increased angiogenesis present in premenopausal patients.[33] We also found significant association between the DD genotype and tumour size, which indicates that ACE level influences the tumour size. This is because this gene is involved in promoting the angiogenesis in cancer cells, high levels of ACE, and increased Ang II promotes angiogenesis and tumour cell proliferation.[34,35] In addition, it has been seen that Ang II leads to increase in the TGF beta 1, platelet-derived growth factor, and basic fibroblast growth factor.[36]

We have found an inverse association between low-activity ACE alleles, ie, II, and breast cancer risk, which supports the fact that renin-angiotensin system is crucially involved in breast carcinogenesis. The two known different receptors[37,38] that bind angiotensin II, known as angiotensin II, ie, type 1 receptor, and angiotensin II, ie, type 2 receptor, are present in epithelial cells of the ducts and lobules in normal mammary tissues, as well as in benign and cancerous tumours of the human mammary tissue.[39,40] Angiotensin II stimulates proliferation in a human breast adenocarcinoma cell line via the angiotensin II type 1 receptor. This proliferation is designated to be involved in the pathogenesis of premalignant lesions.[41] From the activation of angiotensin II type 1 and type 2 receptors, angiotensin II has been shown to induce increased production of nitric oxide,[39] which in turn has been shown to enhance tumour growth and cause metastasis in the murine breast cancer model. Angiotensin II also facilitates metastasis by inducing cell adhesion factors in breast cancer.[42]

In summary, our results suggest that ACE gene polymorphism is associated with breast cancer predisposition and development. The ID genotype may confer a protective effect against breast cancer, and therefore ACE (I/D) polymorphism might be used as a genetic marker for breast cancer risk.

Our results seem conflicting but we acknowledge our results to exposure to different environmental factors and different dietary habits.[43] Therefore, we suggest conducting additional studies to clarify this important association in various other populations. Our results support the evidence that ACE is aetiologically linked to breast cancer development and progression.

Conclusion

The crux of our results is that ID genotype has been suggested to be protective against breast cancer; therefore, the use of ACE inhibitors to control hypertension should be encouraged in place of other hypertensive drugs. The development of chemopreventive agents targeting this angiotensin pathway, to individuals who are at a higher risk for breast cancer, should be used to decrease the incidence of breast cancer.

Acknowledgments

The present research was not funded by any funding agency. The collection of cancer samples used in this study was supported by the Department of Immunology and Molecular Medicines and Department of General Surgery, Sher-I-Kashmir Institute of Medical Sciences.

The authors thank all the breast cancer patients who participated in the study as well as those who are responsible for the creation and maintenance of the entire group in which this study was conducted but were not involved in the current paper.

Authors' contributions

Nidda Syeed formulated, designed and performed the laboratory work for the study, Safiya Abdullah helped in study design, A. Syed Sameer helped in the laboratory work and performed the statistical analysis, Syed Akhtar Husain supervised the study, Sania Nissar and Afshan Rasool helped in the laboratory work and Mushtaq A. Siddiqi coordinated the study, revised the manuscript, and supervised the entire work. All authors have read and approved the final manuscript.

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Source of Support: Nil, **Conflict of Interest:** None declared