Gene Polymorphisms in the Renin-Angiotensin-Aldosterone System and Breast Carcinogenesis: Is There a Connection?

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Clinical Points:

- A positive correlation may exist between polymorphisms of genes that code for proteins of the renin-angiotensin-aldosterone system and a risk of developing breast cancer. These genes code for angiotensin converting enzyme (ACE), angiotensin II receptor type 1 (AGTR1) and angiotensinogen (AGT).
- The polymorphisms in the ACE gene cause variations in the level of ACE and thus the level of angiontensin II in plasma. This increased level of angiotensin II is thought to be one of the determining factors in breast carcinogenesis and therefore long term use of ACE inhibitors in females may prove to be protective against breast cancer.
- The single nucleotide polymorphisms in the AGTR1 gene may reduce the risk of breast cancer by determining the binding efficiency of angiotensin II to the receptor. This receptor may provide a useful target for pharmacological therapy in patients suffering from breast cancer.
- In the future, polymorphisms in ACE, angiotensin II or AGT may prove to be markers for breast cancer.

ABSTRACT
Breast cancer is the second most common type of cancer in the world. In this article, studies are considered, which suggest a pattern may exist between polymorphisms in genes of the renin-angiotensin-aldosterone system (RAAS) and the risk of developing breast cancer. Polymorphisms of angiotensin converting enzyme (ACE), angiotensin II receptor type 1 (AGTR1) and angiotensinogen are investigated. The polymorphisms in the ACE gene cause variations in the level of ACE and therefore affect the level of angiontensin II, which is thought to contribute to breast carcinogenesis. Studies into insertion/ deletion (I/D) polymorphisms and the single nucleotide polymorphism (SNP) A-240T show that carriers of the DD genotype of the I/D polymorphism or the TT genotype of the SNP had significantly greater chances of developing breast cancer than carriers of the II or AA genotypes. SNPs in the AGTR1 gene may reduce the risk of breast cancer by either determining the binding efficiency of angiotensin II to the receptor or by interfering with the downstream signaling cascade that is required for angiotensin II to elucidate it's carcinogenic effect. Studies on the SNPs A-168G, C-535T and T-825A are presented here and it is evident that carriers of the AA genotype, CC genotype and TT genotype may have a higher risk of developing breast cancer than carriers of GG, TT and AA genotypes respectively. A SNP, M-235T, in the angiotensinogen gene also supports the connection of polymorphisms in the RAAS system to breast cancer. This receptor may provide a useful target for pharmacological therapy in patients suffering from breast cancer. In the future polymorphisms in ACE, angiotensin II and AGT may prove to be markers for and long term use of ACE inhibitors may prove to be protective against breast cancer.

INTRODUCTION

After lung cancer breast cancer is the second most common type of cancer worldwide (1) and the fifth most common cause of cancer death (2). Breast cancer is by far the most common cause of cancer incidence and death among women (2) with a global age adjusted incidence of 127.8 per 100,000 annually (3). Like other forms of cancer it is considered to be a multi-factorial event, with both environmental and hereditary factors contributing to the onset of the disease. Such factors include genetic mutations due to oestrogen levels (4), failure of immune surveillance (5) and inherited defects in DNA repair genes such as BRCA1 and BRCA2 (6).

Recent papers have suggested a connection between breast cancer and the renin-angiotensin-aldosterone system (RAAS) (7-10). RAAS is an endocrine system made up of interactions between the metabolites angiotensinogen, angiotensin I, II and III and angiotensin converting enzyme (ACE). This system plays a crucial biological role in the regulaton of vasoconstriction, Na^+ retention, aldosterone and anti-diuretic hormone release (11). The aim of this paper is to review the literature of studies carried out on particular polymorphisms of ACE, angiotensin receptor 1 (AGTR1) and angiotensinogen genes to determine whether a pattern exists between these polymorphisms and the risk of developing breast cancer.

Breast Cancer and the Pattern of ACE Gene Polymorphisms

ACE is a membrane bound dipeptidyl carboxypeptidase which converts angiotensin I to angiotensin II, and is the rate limiting step in the RAAS (11). Three investigators - Koh et al. (12), Ladd et al (13) and Haiman et al (14) - have looked at the patterns of two ACE gene polymorphisms and their possible connections to the risk of developing breast cancer (see Table 1.). The ACE polymorphisms examined were (i) an insertion/deletion (I/D) of a 287 base pair Alu-type sequence in intron 16 and (ii) a single nucleotide polymorphism (SNP) A-240T.

Koh et al., in the Singapore Chinese Health Study, found

Poymorphisms of the ACE gene	Chinese Health Study Koh et al.	Rotherdam Study Ladd et al.	Multi-Ethnic Cohort Study (USA) Haiman et al. Data on all groups
A-240T			
TT	↑↑	↑↑	↔
AT	↑	↑	↑↑
AA	↔	↔	↑
Insetion / Deletion			
DD	↑↑	↑↑	↔
ID	↑	↑	↑
II	↔	↔	↑↑

Table 1. Comparative summary of 3 studies that look at the pattern of polymorphisms found in the ACE gene and the risk of developing breast cancer. The two polymorphisms that were studied were a SNP A-240T and and I/D polymorphism of a 287 base pair Alu-type sequence. ↑↑ : high risk of breast cancer, ↑ : intermediate risk of breast cancer, ↔ : low risk of breast cancer.

that women carrying the polymorphism TT or DD had a significantly higher risk of developing breast cancer than those with the genotype AT or ID while the polymorphisms AA or II carry the lowest risk of breast cancer (12). This result was supported by the Rotterdam study, a case control study on 4,117 women in Holland (13). Ladd et al. found that DD carriers showed a significantly increased risk of developing breast cancer compared to II carriers, an observation that remained even after adjusting for other risk factors such as BMI, age at menarche and menopause and hypertension (see Table. 1). Cancer free survival was also significantly reduced in carriers of the DD polymorphism compared to those with the II polymorphism (13). These studies strongly suggest a link between these polymorphisms and breast cancer. However, Haiman et al in a multiethnic cohort study found conflicting results and concluded that the SNP A-240T and the I/D ACE polymorphisms are not strong predictors of breast cancer risk (see Table 1)(14). When all three studies are considered the results from Koh and Ladd are the most statistically significant. Also the Rotterdam study has the largest sample size and so the evidence is thus in favour of ACE polymorphisms leading to an increase in the risk of breast cancer.

Breast cancer and the pattern of AGTR1 gene polymorphisms

Angiotensin II elicits an effect by binding to both AGTR1 and AGTR2 (7,15). Binding of angiotensin II to AGTR1 stimulates angioneogensis, cell growth and cell proliferation (8,16) while bindingof angiotensin II to AGTR2 causes growth inhibition and cell apoptosis (8,17,18,19). Therefore the effect of angiotensin II on apoptosis depends on the balance of expression of the two receptors on the cell membrane. Increased AGTR1 coupled with decreased AGTR2 expression is observed in breast carcinoma (7,9), laryngeal carcinoma (20), and also squamous cell carcinoma (21) which suggests that its over expression is associated with carcinogenesis.

A follow up study on the Singapore Chinese health study examined the pattern of a number of SNPs in the AGTR1 gene, A-168G, C-535T, T-825A and their connection to breast cancer (22). This study found that the high risk genotypes for breast cancer include the homozygous

SNPs in AGTR1 Gene	Risk of Breast Cancer
A-168G	
AA	↑↑
AG	↑
GG	↔
C-535T	
CC	↑↑
CT	↑
TT	↔
T-825A	
TT	↑↑
TA	↑
AA	↔

Table 2. An overview of three SNPs of the AGTR1 gene and the risk of developing breast cancer. ↑↑ : high risk, ↑ : intermediate risk, ↔ : low risk

alleles AA, CC and TT (see Table 2.). It was also found that the risk of breast cancer was significantly reduced in women who were carriers of the intermediate or low risk SNPs; with an Odds Ratio (OR) of 0.84 (95% confidence intervals (CI) = 0.51-1.37) for women possessing one low risk SNP and an OR of 0.68 (95% CI = 0.46-1.01) for women possessing two/three low risk SNPs (22).

An interesting finding in this study was the additive effect in the reduction of risk of breast cancer that was observed when polymorphisms for both ACE and AGTR1 were considered (see Table 3.). Women possessing the low risk polymorphisms of AGTR1 and ACE had an OR of 0.35 (95% CI = 0.20 – 0.62) of breast cancer risk. This is significantly lower than the breast cancer risk in women who possess only one of these poymorphisms. This observation lends further weight to the argument that gene variations within the RAAS may play a role in breast carcinogeneis.

Breast Cancer and the Pattern of Angiotensinogen Gene Polymorphisms

Angiotensinogen is the precursor of angiotensin I in RAAS.

AGT1R Poylmorphisms	ACE Polymorphisms High Risk	Low Risk
	OR (95% CI)	OR (95% CI)
High risk	1	0.46 (0.28 – 0.76)
Low risk	0.57 (0.25 – 1.32)	0.35 (0.20 – 0.62)

Table 3. An overview of the high or low risk combinations of AGT1R and ACE polymorphisms and their risk of developing breast cancer. OR=odds ratio, CI = confidence interval.

Ladd et al. investigated a possible link between the pattern of the SNP, M-235T, in the angiotensinogen gene and breast cancer risk in caucasian postmenopausal women and found that women carrying the MM genotype were more likely to have breast cancer in comparison to the MT and TT genotypes (see Table 4.) (23).

SNP in Angiotensinogen gene	Risk of Breast Cancer
M-235T	
MM	↑↑
MT	↑
TT	↔

Table 4. An overview of the variable AGT polymorphisms and the risk of developing breast cancer. ↑↑ : high risk of breast cancer, ↑ : intermediate risk of breast cancer, ↔ : low risk of breast cancer.

DISCUSSION

Polymorphisms in the the RAAS system have an established association with cardiac disease (11). In this review, we were interested in examining the possible link between gene polymorphisms in three proteins of the RAAS system, ACE, AGTR1 and angiotensinogen and breast cancer. The review of literature suggests a positive correlation exists between polymorphisims in these genes and a risk of breast cancer but one must wonder by what mechanism do these polymorphisms result in the development of breast cancer and what are the future implications of such a connection?

1) How do polymorphisims in ACE lead to an increase in breast cancer risk?

According to the studies carried out by Koh et al (12) and Ladd et al (13) both polymorphisms of ACE, the insertion or deletion of a 287 base pair Alu-type sequence and an A-240T SNP, may lead to an increased risk of breast cancer by affecting the level of ACE produced *in vivo*. If ACE is increased in the plasma there is an increase in the production of angiotensin II which is thought to be the direct cause of breast cancer via the binding to its receptors AGTR1 and AGTR2.

Studies into the ACE levels in plasma have shown that they vary between 28 and 47% depending upon the insertion or deletion of the Alu-type sequence (12,24,25). The SNP A-240T also results in variations of ACE levels in the plasma, with the homozgous genotype TT resulting in the highest level of ACE, the homozygous genotype AA resulting in the lowest level of ACE and the TA genotype resulting in levels of an intermediate level. These patterns of fluctuating ACE levels are consistent with the prevalence of breast cancer risk.

Haiman et al. (14) in their multiethnic cohort study concluded that A-240T and I/D ACE polymorphisms are not likely to be strong predictors of breast cancer risk (see Table 1.). However, this finding may be due to the fact that it was a US based study and it is well recognised that the effect of a given gene on disease risk is masked in racially mixed populations (26,27). The studies of Koh et al. and Ladd et al. were performed in Singapore and the Netherlands respectively, countries which have much more homogenous populations than the US and therefore would not have had the risk of masking of the genetic effect on the disease. Diet derived long chain polyunsaturated fatty acids and tea polyphenols have been shown to modulate the effects of angiotensin II on the cardiovascular system (22) and Koh et al used this observation to offer another explanation for the conflicting data of the Haiman et al. study when they pointed out that differences in diet, especially between the populations in Singapore and the US, may exert an influence on the effect of angiotensin II on breast carcinogenesis.

2) How do polymorphisims in AGTR1 lead to an increase in breast cancer risk?

Angiotensin II appears to exert a carcinogenic effect via the AGTR1 receptor via three different pathways by: i) inducing cell division via regulation of mitogenic signalling pathways achieved by transactivation of protein kinase C and epidermal growth factor receptor (28), ii) angioneogenesis and promoting arterial smooth muscle cell proliferation via vascular endothelial growth factor from nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (8,16,29) and iii) production of reactive oxygen species from NADPH oxidase (30,31)

The SNPs A-168G, C-535T, T-825A all decreased the risk of breast cancer (22) which suggests that a variation in the receptor AGTR1 does not allow angiotensin to bind correctly and initiate the carcinogenic intracellular signaling pathway. It was also found that those people who had the combined low risk genotypes of AGTR1 and ACE had an even lower risk of breast cancer when compared to carriers of only one. Further evidence that angiotensin II binding to AGT1R is involved in breast cancer has been the observation losartan prevents the proliferative effects of angiotensin II in vitro and in animal models (7,28).

3) How do polymorphisims in AGT lead to an increase in breast cancer risk?

It is important to point out that the study of genetic polymorphisms in AGT is not as straightforward as the study of genetic polymorphisms in ACE or AGTR1. While an increase in AGT does lead to an increased level of angtiotensin II which is thought to be a carcinogenic factor, AGT does have antiangiogenic actions such as reducing endothelial cell proliferation and migration (32). Ladd et al. found that women carrying the MM genotype of the M235T AGT polymorphism had an increased risk of developing

breast cancer when compared to those women carrying the MT and TT genotypes. The authors felt that the findings suggested that the antiproliferative effects of AGT may override the proliferative effects of angiotensin II. It is important to note that the conclusion of the above study is controversial and further studies on the subject are required before any firm conclusions may be drawn.

4) The Future - ACE inhibitors in breast cancer ?

If a connection is established between genetic polymorphisms in the ACE gene, ACE plasma levels and the risk of breast cancer, then it may be possible to use ACE-inhibitors (ACE-I) not only as an antihypertensive but also as an anti-carcinogenic therapy. Lever et al. created much excitement with their paper when they found that hypertensive patients on ACE-I had a lower risk of cancer versus population controls, the lowest being breast cancer (10). Other antihypertensive treatments had no apparent effect on risk of cancer and the authors concluded that long-term use of ACE-I may have protective properties against cancer. However numerous negative studies such as the one from Fryzek et al. do raise a significant point that the prevention of breast carcinogenesis with ACE-I may only be beneficial in patients with an increased genetic expression of ACE and AGT1R levels (33). While not suggesting that ACE-I should be given prophylactically to prevent breast cancer, it may be viewed favourably when choosing a class of antihypertensive medication. The relationship may indeed be likened to the protective role of low dose aspirin and colorectal carcinoma. The new knowledge of these potential benefits of ACE-I in breast cancer coupled together with their known potency in lowering morbidity and mortality in hypertension helps to strongly suggest ACE-I as a first line treatment in hypertensive individuals and perhaps more so in women.

CONCLUSION

Polymorphisms in the genes encoding proteins of the RAAS system examined ie. ACE, AGTR1 and AGT, may play an important role in determining the risk of breast cancer. It is thought that these polymorphisms may provide markers for breast cancer in the future, although more detailed trials and experiments are required to confirm this conclusion. Also, it may be possible that pharmacological inhibition of the angiotensin II carcinogenic effect, by inhibition of either ACE or AGTR1, could be used to prevent or treat breast cancer.

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