International Journal of Research
Available at https://edupediapublications.org/journals
p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 03 Issue 11
July 2016

Study of ATM and Chek2 Genes SNPS and Their Association with Breast Cancer

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Abstract:

To analyze ATM and CHEK2 genes for the presence of SNPs and their association with breast cancer. Breast cancer is a malignant tumour that starts in the cells of the breast. 10-15% of breast cancer cases have some family history of the disease, only 5% can be explained by rare, highly penetrant mutations in genes such as *BRCA1* and *BRCA2* (First-degree relatives of breast cancer patients have a 2-fold increase in risk over the general population). Breast cancer can be separated into different types based on the way the cancer cells look under the microscope. Risk factors could be genetic or environmental, or in most cases, a combination of the two. The higher rate of most breast cancers in monozygotic twins of case patients than in dizygotic twins or siblings suggests that most familial clustering is the result of inherited genetic factors rather than lifestyle or environmental factors. Some of this clustering can be explained by mutations in specific genes that confer high risk of disease. However, such susceptibility alleles are rare in the population. For example, highly penetrant variants in the breast cancer susceptibility genes *BRCA1* and *BRCA2* account for less than 20% of the total genetic risk of breast cancer and other, rarer high-penetrance genes such as *TP53* and *PTEN* account for less than 5% of the risk. It is likely that much of the unexplained familial risk is due to alleles of low to moderate penetrance. The ability to identify such genetic variants can be further improved by careful selection of both candidate gene and candidate polymorphism. The study involves 50 breast cancer cases and 50 control subjects. The SNP 5144 A>T in ATM promoter was examined by PCR-RFLP using FokI restriction enzyme and gel electrophoresis. The SNP 1100del C>G in CHEK 2 was examined by allele specific PCR followed by gel electrophoresis. The genotypes were determined and compared between patients and controls and the effect of polymorphism on clinicopathological data of breast cancer patients was studied. The frequencies of del/C and del/del genotypes of CHEK2 delC polymorphism and AT and T allele of ATM -5144A>T polymorphism had shown an elevation with respect to clinical variables of breast cancer such as tumor stage, tumor type and ER/PR status even though there was no significant difference in the genotype distribution between cases and controls.

Keywords: ATM -5144A>T, *BRCA1* and *BRCA2*, PCR-RFLP, *TP53* and *PTEN*.

INTRODUCTION

Breast cancer is the second most common cancer in the rest of the world. Estrogens and Insulin-like growth factor-1 (IGF-1) are involved not only in development of normal breasts but also in breast carcinogenesis.

Disease-causing mutations have been found in several genes, most notably in *Breast Cancer 1* and *2* (*BRCA1* and *BRCA2*). A substantial proportion of breast cancer risk may be the result of a combination of genetic and lifestyle factors. Genetic polymorphisms in genes coding for growth factors and genes involved in estrogen and drug metabolism may modify not only breast cancer risk but also prognosis and early recurrences. Cancer arises as a result of a number of genetic alterations in the dividing cell. The hallmarks of cancer are cells with limitless cell-dividing capacity that are self-sufficient in growth factors and insensitive to anti-growth signals, and with the ability to evade apoptosis, the programmed cell death by which defective cells are usually eliminated. The tumor cells also acquire the ability to invade tissue, and are then referred to as invasive cancer. These cells are dependent on nutrients and oxygen supplied by the blood, and must therefore be able to create new blood vessels (sustained angiogenesis). The most aggressive form of cancer can penetrate the blood vessels and the lymphatic system, giving rise to metastases in other parts of the body.

Hereditary breast cancer accounts for only 5-10% of all breast cancers and germline mutations with the two major breast cancer susceptibility genes *BRCA1* and *BRCA2*, being responsible for a small fraction (~2-3%) of all breast cancers. In addition to *BRCA1* and *BRCA2*, *TP53* and *PTEN* are considered to be high-penetrance breast cancer susceptibility genes, whereas *ATM*, *BRIP1*, *CHEK2*, and *PALB2* are considered to be moderate-penetrance susceptibility genes (Liaw *et al*, 1997; Stratton *et al*, 2008). A large proportion of familial aggregation of breast cancer, and possibly non-familial disease, is considered to be due to the effect of low-risk alleles, some being very common and possibly acting via polygenic mechanisms and in interaction with environmental and lifestyle factors. Breast cancer can be separated into different types based on the way the cancer cells look under the microscope.

Most breast cancers are *carcinomas* are often a type of carcinoma called *adenocarcinoma*, which is carcinoma that starts in glandular tissue. Other types of cancers can occur in the breast, too, such as sarcomas, which start in the cells of muscle, fat, or connective tissue. Breast cancer can also be classified based on proteins on or in the cancer cells, into groups like hormone receptor-positive or triple-negative. Breast cancer is primarily treated with surgery, either by means of modified radical mastectomy, whereby the complete breast is removed, or by means of breast-conservative surgery, whereby only part of the tissue is removed. During surgery axillary lymph nodes are removed, in many cases only the sentinel node. In active surgery, only part of the tissue is removed. Adjuvant therapy improves the prognosis for many patients, but this advantage should be considered in relation to side effects. Adjuvant therapies for breast cancer patients include radiation therapy (RT), chemotherapy, endocrine therapy and antibodies, and are chosen based on prognostic and treatment predictive factors. Combinations of these adjuvant regimes are often used. Neoadjuvant treatment is administered in order to decrease tumor size prior to surgery, and to facilitate the evaluation of treatment response.

It is estimated that approximately 20% of drug therapies are influenced by genetic polymorphisms in drug-metabolizing genes. Each individual is unique, though

comparison of the genomes of any two individuals only shows a ~0.1% difference. Single nucleotide polymorphisms (SNPs) explain up to 95% of all variant DNA sites (Meyer, 2004). SNP is by definition a nucleotide exchange that occurs in at least one percent of a population. Genetic variant that occurs in less than 1% of a given population is referred to as a mutation or rare variant. These alterations arise somatically at a high rate, particularly in cancer cells where they might become enriched, but they may also occur in germ cells, and can thus be transmitted as constitutional variants to coming generations. Various DNA repair mechanisms normally act to preserve high genome integrity, but never with complete fidelity. Sequence alterations can take place anywhere in the genome, and the vast majority end up in non-coding sequences and have no or little effect on cell function. Few SNPs located in the promoter or functionally important coding regions of candidate genes play important role in cancer pathogenesis and prognosis.

ATM: ATM gene is localized to the long arm of chromosome 11 at a position 11q22-q23. It is composed of 66 exons (62 of which are coding) and covers approximately 150 kb of genomic DN (7). The ATM gene helps repair damaged DNA. Inheriting two abnormal copies of this gene causes the disease ataxia-telangiectasia. Inheriting one abnormal ATM gene has been linked to an increased rate of breast cancer in some families.

CHEK2: The tumor suppressor gene CHEK2 maps to the long arm of chromosome 22 at position 22q12.1 and consists of 14 coding exons. CHEK2 plays a significant role in the regulation of the cell response in the presence of DNA double strand breaks. It carries out the control at all major checkpoints of the cell cycle, regulates the repair process and directs the cell to apoptosis [40]. ATM is a main activator of CHEK2 and its activation is accomplished by phosphorylation of Thr68 in the SCD domain. According to the Ensemble database a total of 1924 alterations in human CHEK2 gene are so far registered. Most of them are localized in non-coding sequences while others are scattered in the coding gene regions without formation of “hot spots”. It is established that among the mutations found in CHEK2 in patients with breast cancer deletions, substitutions or insertion of single nucleotides are dominating. Most of the mutations are germ-line, but also somatic mutations are found, though with considerably lower frequency

International Journal of Research
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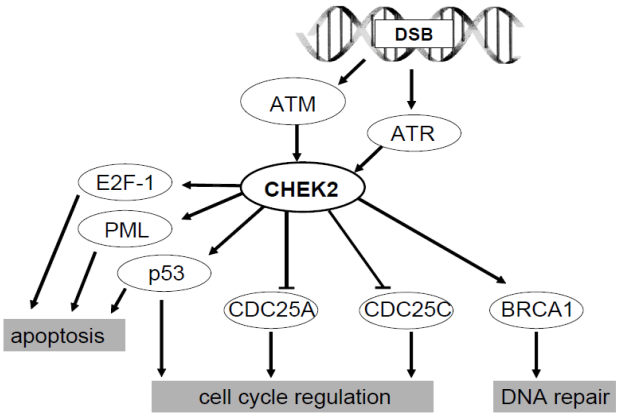

MATERIALS AND METHODS:

SOURCE OF SAMPLING& STUDY DESIGN

For the present study, Breast cancer cases were recruited from Nizam's Institute of Medical Sciences, Hyderabad. 5ml blood sample was collected from 50 patients into EDTA vaccutainers which were used for DNA isolation through non-enzymatic/salting out method. SNPs were analyzed through ARMS (Amplification refractory mutation system)-PCR and RFLP (Restriction Fragment Length Polymorphism) methods followed by agarose gel electrophoresis. 50 age matched females were recruited from the local population for the case-control comparison. GENOTYPING OF CHEK2 POLYMORPHISM BY ARMS-PCR METHOD ARMS technique for detecting known point mutations was first described by Newton et al.

PRINCIPLE:

PCR was based on analysing known point mutations in DNA and distinguishing between the normal, heterozygous and homozygous mutant genotypes. ARMS –PCR is based on the principle that a mismatch between the 3`nucleotide of a PCR primer and the template reduces or prevent primer extension by Tag polymerase. A variety of strategies have been developed using primers that are complementary to allow for the specific amplification of individual alleles. This method is most commonly referred to as a PCR-ARMS or ARMS and it also been referred to as PASA(PCR Amplification of specific Alleles) by sommer et al in 1989 and ASPCR(Allele specific PCR)by Wu et al. in 1989. Design and optimization of ARMS-PCR is primarily a function of the target sequence and the nucleotide differences that defines the alleles. In addition to the mismatches between 3` terminal base of the primer and the target, single mismatch should be incorporated at a several positions from the 3` terminus. Primers have comparable theoretical melting temperature(Tm).

RESULTS

In the current case-control genetic association study, 50 breast cancer cases and 55 controls were analysed for ATM and CHEK2 SNPs and assessed for genetic association with breast cancer development comparing with respect to epidemiological

and clinical risk variables. The following the distribution of cases for each variable:

Following are the genotype and allele distributions among cases and controls for the SNPs analysed.

Table1.1: Genotype frequency of the ATMP -5144 A>T gene polymorphism in breast cancer cases and controls

	No: of individuals (%)			
ATMP-5144 A>T	**Controls (N=55)**	**Breast cancer (N=50)**	**OR (95% CI)**	**χ2 p**
[a] Codominant model				
AA	40(72.7%)	33(60%)	**1.00(Ref)**	0.79
AT	14(24.5%)	14(28.0%)	1.21(0.51-2.90)	
TT	1(1.8%)	3(6.0%)	3.63(0.36-36.6)	
[b] Dominant model				
AA	40(72.7%)	33(60.0%)	**1.00(Ref)**	0.45
AT+TT	15(27.3%)	17(40.0%)	1.37(0.59-3.16)	
[c] Recessive model				
AA+AT	54(98.2%)	47(94.0%)	**1.00(Ref)**	
TT	1(1.8%)	3(6.0%)	3.44(0.34-34.2)	0.54
[d] Over dominant model				
AA+TT	41(74.5%)	36(72.0%)	**1.00(Ref)**	0.76
AT	14(25.5%)	14(28.0%)	1.13(0.47-2.70)	
Allele				
A	94(85.5%)	80(80.0%)	**1.00(Ref)**	0.29
T	16(14.5%)	20(20.0%)	1.46(0.71-3.02)	
HWE (p)	**0.03**	**0.78**		
*p<0.05; #p<0.10(χ2 p values)				

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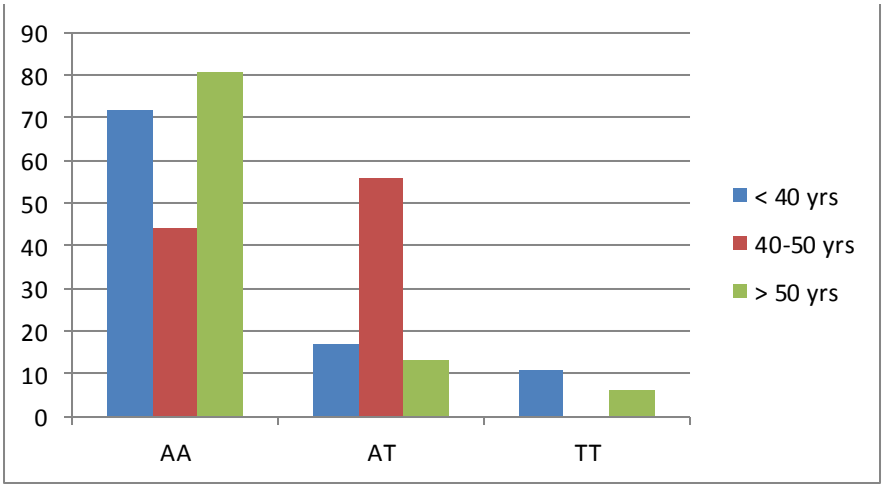
Inference: Heterozygous mutant AT genotype and variant TT genotype frequencies of ATMP -5144 A>T polymorphism were elevated in breast cancer patients under co-dominant, dominant, recessive as well as over dominant model and revealed increased risk for breast cancer development.

Table1.2: Genotype distribution of ATM -5144 A>T gene polymorphism with respect to Age at Onset

Age	Genotype frequencies							Allelic Frequencies	
	AA		AT		TT		Total	A	T
	n	%	N	%	n	%			
< 40 years	13	72	3	17	2	11	18	0.81	0.19
40-50 years	7	44	9	56	0	0	16	0.72	0.28
>50 years	13	81	2	13	1	6	16	0.88	0.12
AA vs AT: AT vs TT: OR (95% CI) - 5.5714 (1.1278 to 27.523); OR(95%CI)-0.07(0.002-1.94) OR (95% CI) -0.770 (0.0152 to 8.527) ; OR(95%CI)-0.75(0.03-14.97) AA vs TT: OR (95% CI) - 0.6667 (0.0951 to 4.6736) OR (95% CI) – 0.500 (0.0402 to 6.2180) Yates p value :0.144 : d.f 4									



International Journal of Research
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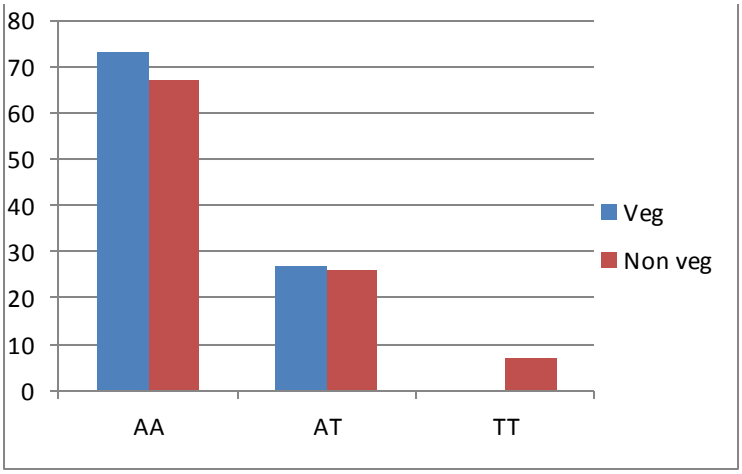
Inference: Frequency of AT genotype increased in individuals in age group 40-50years (56.0%) than individuals who are in age group >50 years (13.0%), indicating that the disease is more prone in AT genotype carriers who are in the age group <40years.

Table1.3: Genotype distribution of ATM-5144A>T polymorphism with respective to diet

Diet	Genotype frequencies							Allelic Frequencies	
	AA		AT		TT		Total	A	T
	n	%	N	%	n	%			
Veg	8	73	3	27	0	0	11	0.68	0.32
Non veg	26	67	10	26	3	7	39	0.79	0.21
AA vs AT: OR (95% CI) – 0.76 (0.1888 to 3.1333) AA vs TT : OR (95% CI) – 2.2453 (0.1050 to 47.9970) AT vs TT : OR(95%CI)- 2.33(0.09-57.28) Yates p value : 0.94 , d.f -2									



Inference: No significant association was observed between ATM-5144A>T polymorphism with respect to diet of breast cancer patients.

Table1.4: Genotype distribution of ATM-5144 A>T polymorphism with respect to menopausal status

Menopausal status	Genotype frequencies							Allelic Frequencies	
	AA		AT		TT		Total	A	T
	n	%	N	%	n	%			

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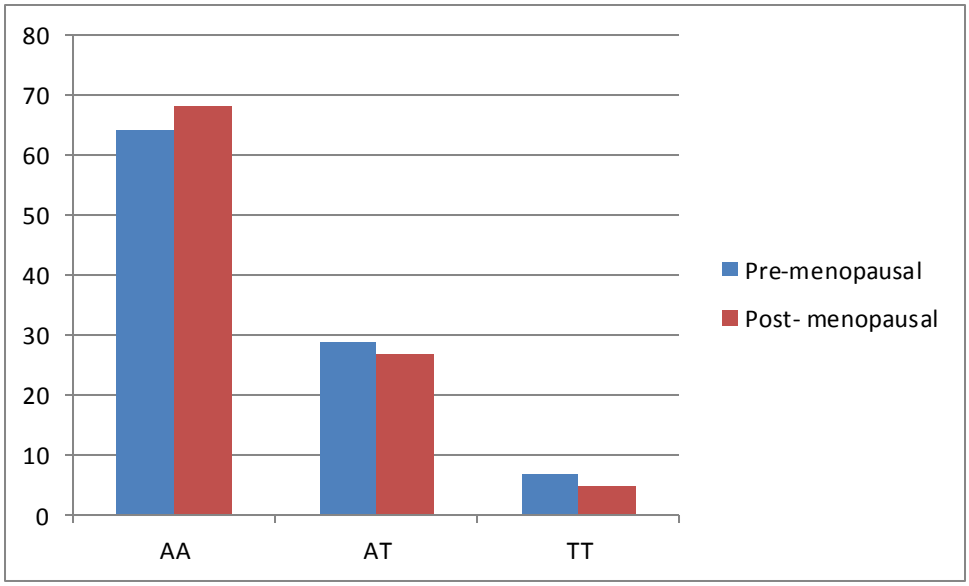
Pre-menopausal	18	64	8	29	2	7	28	0.79	0.21
Post- menopausal	15	68	6	27	1	5	22	0.82	0.18
AA vs AT: OR (95% CI) – 0.90 (0.2550 to 3.1759) AA vs TT : OR (95% CI) – 0.60 (0.0494 to 7.2831) AT vs TT : OR(95%CI) -0.66(0.04-9.18) Yates p value : 0.96; d.f 2									

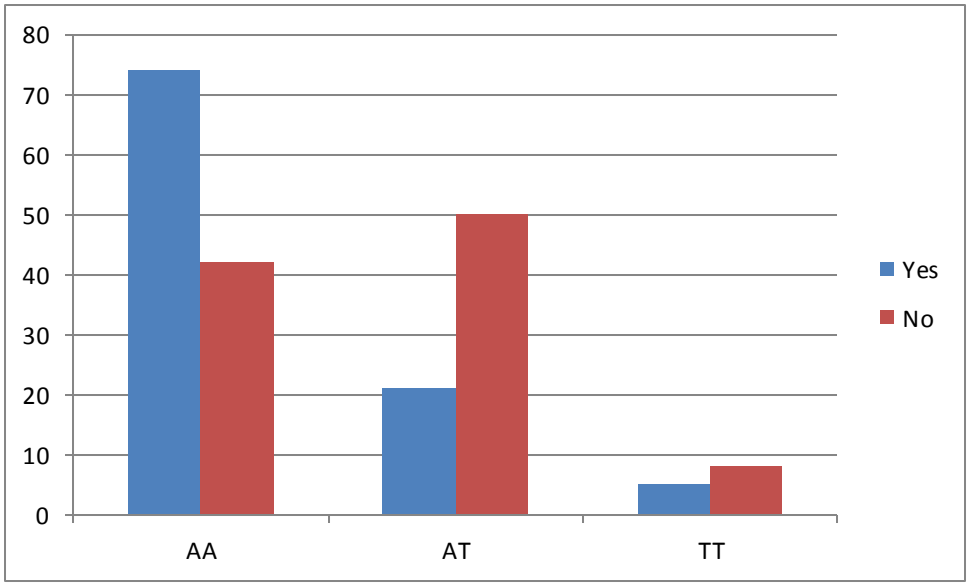


Inference: No significant association was observed between ATM-5144A>T polymorphism with respect to menopausal status among breast cancer patients.

Table1.5: Genotype distribution of ATM-5144 A>T polymorphism with respect to Lactation

Lactation	Genotype frequencies							Allelic Frequencies	
	AA		AT		TT		Total	A	T
	N	%	N	%	n	%			
Yes	28	74	8	21	2	5	38	0.82	0.18

No	5	42	6	50	1	8	12	0.67	0.33
AA vs AT: OR (95% CI) – 4.20 (1.0118 to 17.4346) AA vs TT: OR (95% CI) – 2.80 (0.2117 to 37.0345) AT vs TT: OR (95% CI)-0.66(0.04-9.19) Yates p value : 0.23 , d.f 2									

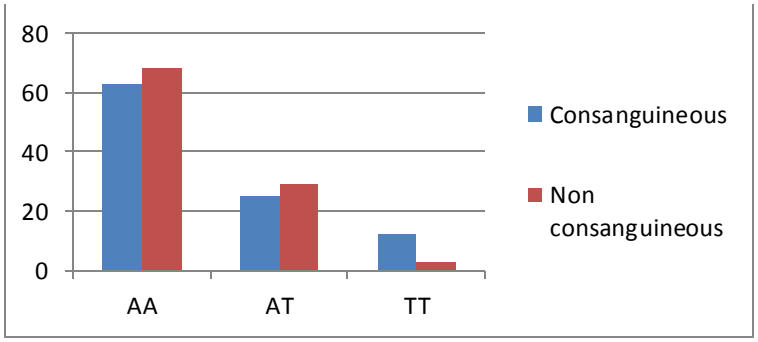


Inference: AT (50%) and TT genotype (8%) frequencies of ATM-5144 A>T polymorphism are elevated in Non-breastfeeding patients compared to breast feeding patients.

Table1.6: Genotype distribution of ATM-5144 A>T polymorphism with respect to consanguinity

Consanguinity	Genotype frequencies							Allelic Frequencies	
	AA		AT		TT		Total	A	T
	n	%	N	%	n	%			
Consanguineous	10	63	4	25	2	12	16	0.75	0.25
Non consanguineous	23	68	10	29	1	3	34	0.82	0.18

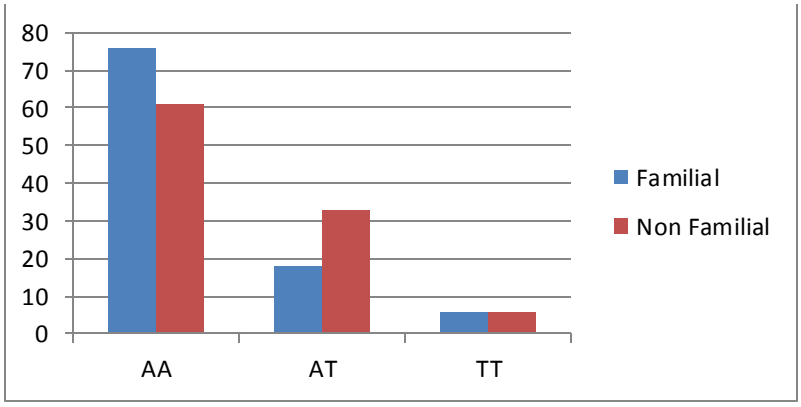
AA vs AT: OR (95% CI) – 1.087 (0.2743 to 4.3070)
AA vs TT : OR (95% CI) – 0.217 (0.0176 to 2.6822)
AT vs TT : OR(95%CI)-0.20(0.01-2.87)
Yates p value : 0.799; d.f. 2



Inference: With respect to consanguinity, TT genotype was increased (12.0%) among consanguineous breast cancer. It was observed that T allele frequency of ATM-5144A>T polymorphism elevated in consanguineous patients compared to Non-consanguineous patients.

Table1.7: Genotype distribution of ATM-5144 A>T polymorphism with respect to Familial incidence

Familial incidence	Genotype frequencies							Allelic Frequencies	
	AA		AT		TT		Total	A	T
	n	%	N	%	n	%			
Familial	13	76	3	18	1	6	17	0.85	0.15
Non Familial	20	61	11	33	2	6	33	0.77	0.23
AA vs AT: OR (95% CI) – 2.383 (0.5562 to 10.2125) AA vs TT: OR (95% CI) – 1.300 (0.1067 to 15.8372) AT vs TT: OR(95%CI)-0.54(0.03-8.27) Yates p value : 0.58;d.f-2									



Inference: With respect to Familial incidence, the heterozygote AT genotype (18.0%) and T allele (15.0%) frequencies are reduced among familial breast cancer cases.

Table1.8: Genotype distribution of ATM-5144 A>T polymorphism with respect to Area of living

Area of living	Genotype frequencies							Allelic Frequencies	
	AA		AT		TT		Total	A	T
	n	%	N	%	n	%			
Urban	18	90	2	10	0	0	20	0.95	0.05
rural	15	50	11	37	4	13	30	0.68	0.32
AA vs AT: OR (95% CI) – 6.600 (1.2610 to 34.5440) AA vs TT :OR (95% CI) – 10.741 (0.5355 to 215.4839) AT vs TT: OR(95%CI) -1.95(0.07-49.26) Yates p value : 0.051; d.f-2									

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e-ISSN: 2348-795X
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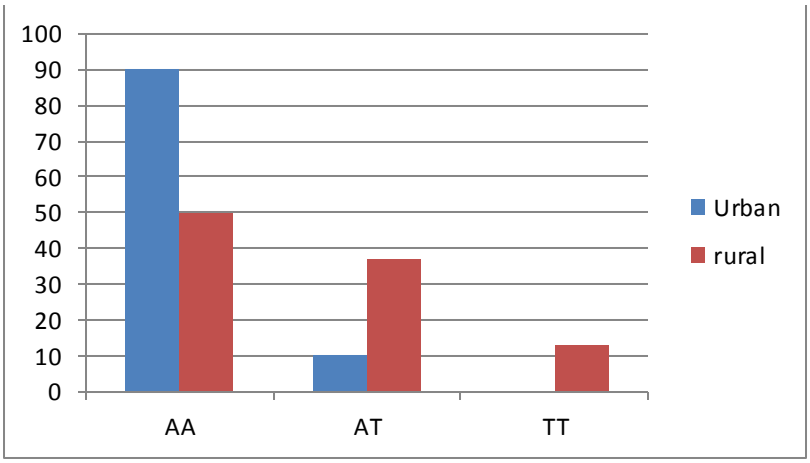
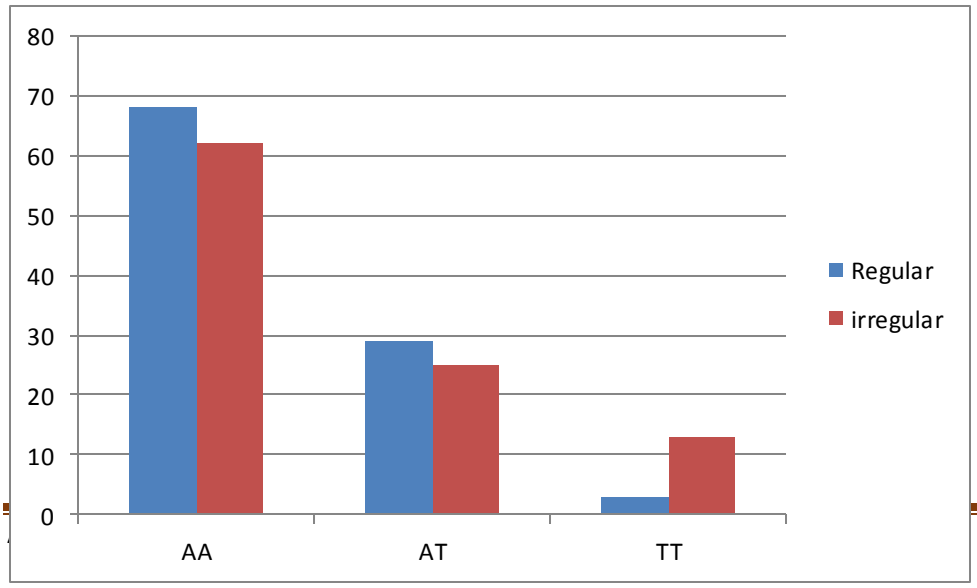


Inference: With respect to area of living, the frequencies of AT genotype (37.0%) and T allele (32.0%) are elevated among patients from rural area.

Table1.9: Genotyping distribution of ATM-5144A>T polymorphism with respect to Menstrual cycle

Menstrual cycle	Genotype frequencies							Allelic Frequencies	
	AA		AT		TT		Total	A	T
	n	%	N	%	n	%			
Regular	23	68	10	29	1	3	34	0.82	0.18
Irregular	10	62	4	25	2	13	16	0.75	0.25



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p-ISSN: 2348-6848
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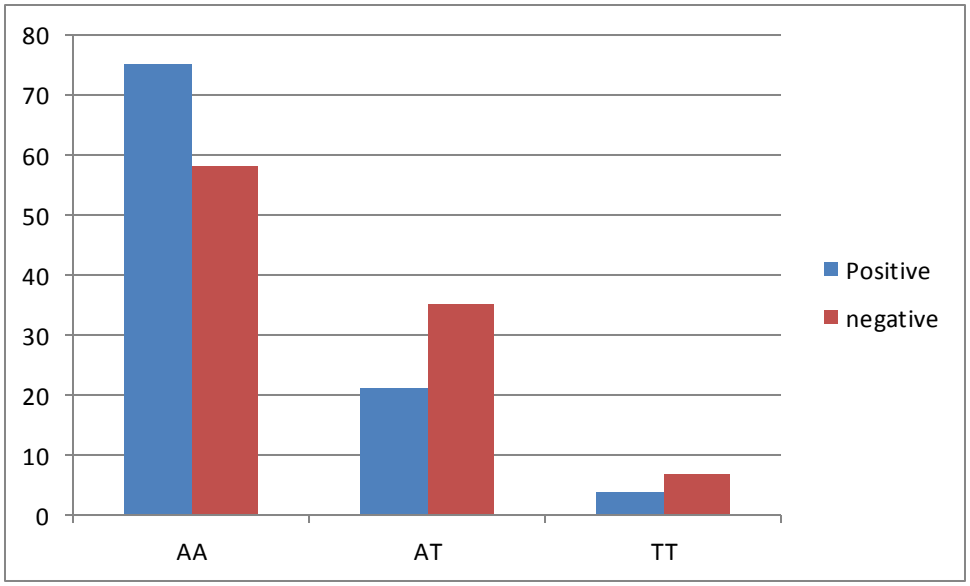
Inference: The TT genotype frequency (13%) of ATM-5144A>T polymorphism was elevated in patients with irregular history of menstruation. Similarly, T allele frequency had shown slight elevation among BC patients with irregular history of menstruation compared to regular history of menstruation.

Table1.10: Genotype distribution of ATM-5144 A>T polymorphism with respect to smoking status

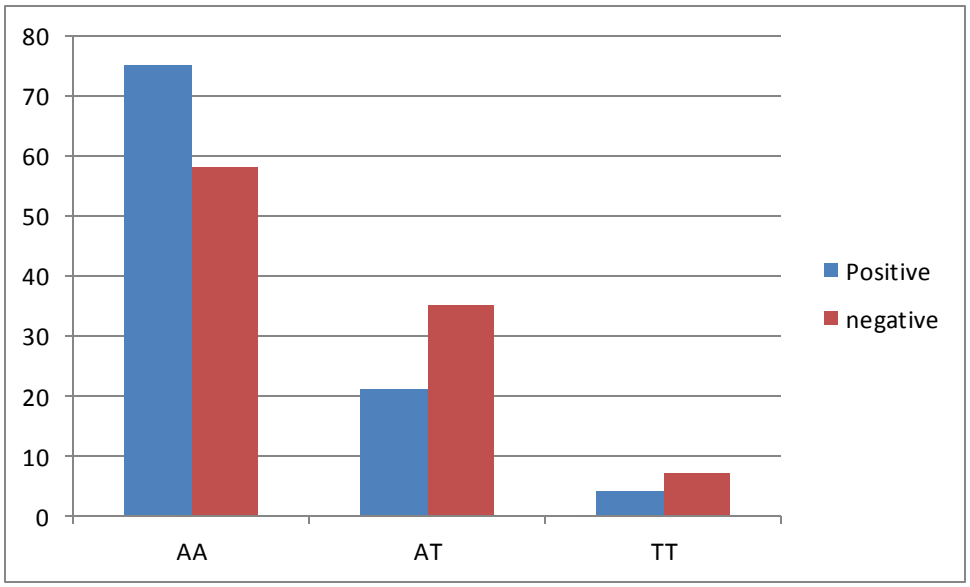
Habits	Genotype frequencies							Allelic Frequencies	
	AA		AT		TT		Total	A	T
	n	%	N	%	n	%			
Smoking	13	72	3	17	2	11	18	0.81	0.19
Non smoking	20	63	11	34	1	3	32	0.70	0.30
AA vs AT : OR (95% CI) – 2.38 (0.55 to 10.21) AA vs TT: OR (95% CI) – 0.32 (0.02 to 3.95) AT vs TT: OR(95%CI) -0.13(0.009-2.06) Yates p value : 0.594; d.f-2									



Inference: AT genotype frequency was increased (34%) among patients with no positive smoking history. It was observed that T allele frequency was also elevated among non smokers compared to smokers.

Table1.11: Genotype distribution of ATM -5144A>T polymorphism with respect to Estrogen receptor

Estrogen receptor	Genotype frequencies							Allelic Frequencies	
	AA		AT		TT		Total	A	T
	n	%	N	%	n	%			
Positive	18	75	5	21	1	4	24	0.85	0.15
Negative	15	58	9	35	2	7	26	0.75	0.25
AA vs AT:OR (95% CI) – 2.160 (0.5944 to 7.8486) AA vs TT:OR (95% CI) – 2.40 (0.1977 to 29.1324) AT vs TT: OR(95%CI)-1.11(0.07-15.53) Yates p value 0.682;d.f-2									



Inference: AT genotype was increased (35%) among patients with ER negative status. It was observed that T allele frequency of ATM-5144A>T polymorphism elevated in ER-ve status compared to ER positive status.

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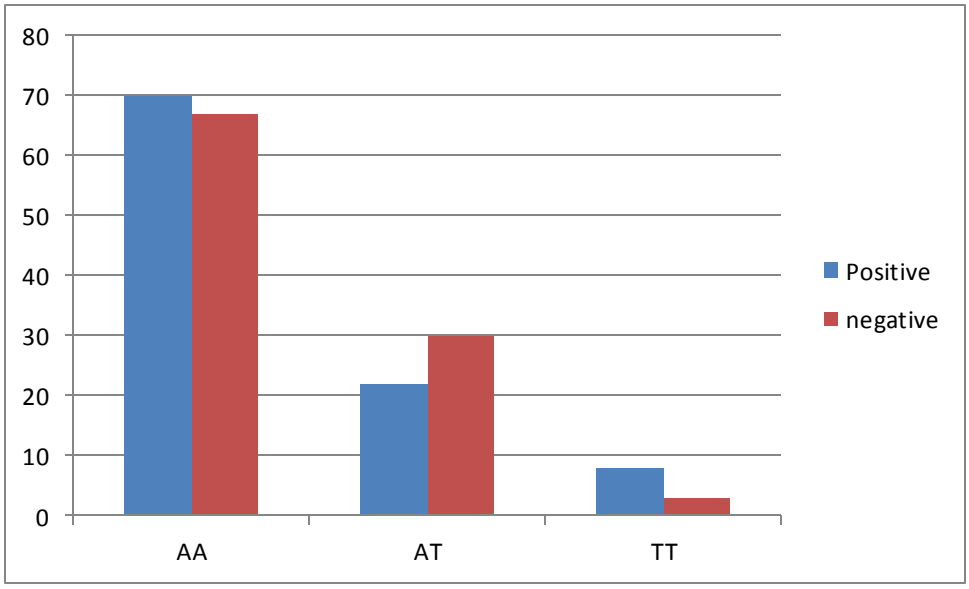
Table1.12: Genotype distribution of ATM -5144 A>T polymorphism with respect to Progesterone receptor

Progesterone receptor	Genotype frequencies							Allelic Frequencies	
	AA		AT		TT		Total	A	T
	n	%	N	%	n	%			
Positive	16	70	5	22	2	8	23	0.80	0.20
Negative	18	67	8	30	1	3	27	0.81	0.19
AA vs AT:OR (95% CI) – 1.42 (0.38 to 5.24) AA vs TT:OR (95% CI) – 0.44 (0.03 to 5.37) AT vs TT:OR (95%CI)-0.31(0.22-4.41) Yates p value : 0.945;d.f-2									



Inference: No significant association was observed between ATM-5144A>T polymorphism and Progesterone receptor status.

International Journal of Research
Available at https://edupediapublications.org/journals
p-ISSN: 2348-6848
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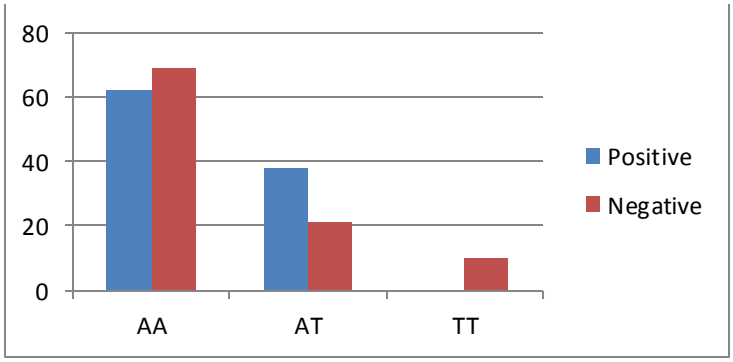
Table1.13: Genotype distribution of ATM-5144 A>T polymorphism with respect to HER2 status

HER 2 status	Genotype frequencies							Allelic Frequencies	
	AA		AT		TT		Total	A	T
	n	%	N	%	N	%			
Positive	13	62	8	38	0	0	21	0.81	0.19
Negative	20	69	6	21	3	10	29	0.79	0.21
AA vs AT: OR (95% CI) – 0.48 (0.13to 1.76) AA vs TT:OR (95% CI) – 4.60(0.22 to 96.54) AT vs TT :OR(95%CI)-9.15(0.39-21.28) Yates p value : 0.454;d.f-2									

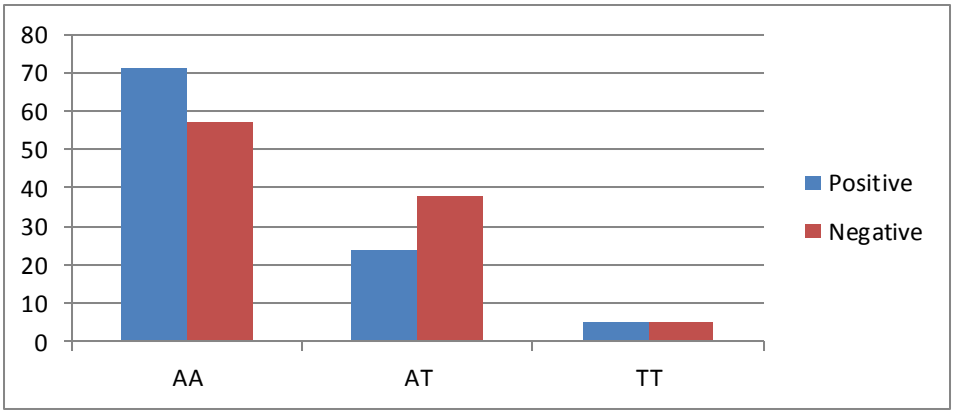


Inference: No significant association was observed for the ATM-5144A>T polymorphism with respect to HER2 receptor status.

Table1.14: Genotype distribution of ATM -5144 A>T polymorphism with respect to Axillary Node

Axillary Node	Genotype frequencies							Allelic Frequencies	
	AA		AT		TT		Total	A	T
	n	%	N	%	n	%			

Positive	24	71	8	24	2	5	34	0.82	0.18
Negative	9	57	6	38	1	5	16	0.75	0.25
AA vs AT:OR (95% CI) – 2.00 (0.54to 7.38) AA vs TT:OR (95% CI) – 1.33 (0.10 to 16.56) AT vs TT:OR(95%CI)-0.66(0.04-9.18) Yates p value : 0.662; d.f-2									



Inference: No significant association was observed between ATM-5144A>T polymorphism and with respective to Axillary lymph node.

Table1.15: Genotype distribution of ATM-5144 A>T polymorphism with respect to Tumor Stage

Tumour stages	Genotype frequencies							Allelic Frequencies	
	AA		AT		TT		Total	A	T
	n	%	N	%	n	%			
Early stage	15	63	7	29	2	8	24	0.77	0.23
Advanced stage	18	69	7	27	1	4	26	0.83	0.17

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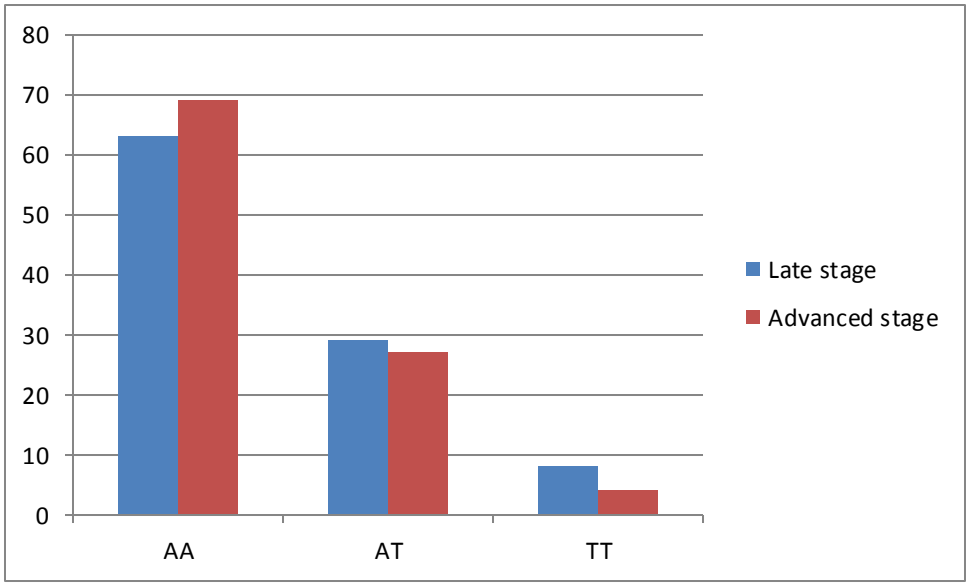
AA vs AT:OR (95% CI) – 0.83 (0.23 to 2.91)
AA vs TT:OR (95% CI) – 0.41(0.03 to 5.05)
AT vs TT:OR(95%CI)-0.50(0.03-6.86)
Yates p value : 0.9704;d.f-2

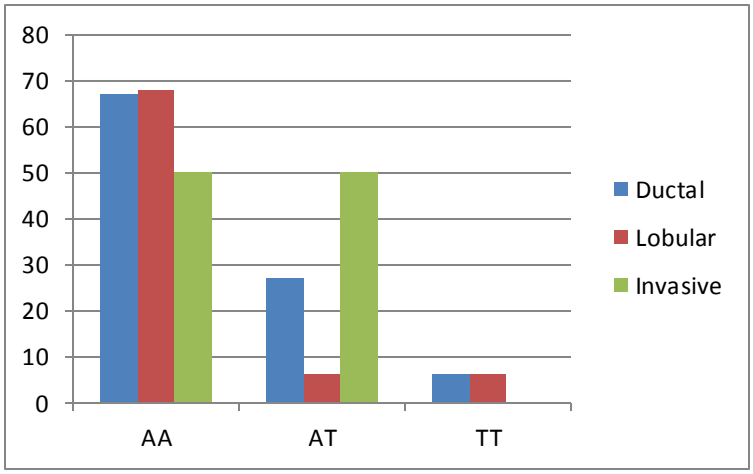


Inference: No significant association was observed between ATM-5144A>T polymorphism and stage of breast cancer.

Table1.16: Genotype distribution of ATM -5144 A>T polymorphism with respect to Type of breast cancer

Type	Genotype frequencies							Allelic Frequencies	
	AA		AT		TT		Total	A	T
	n	%	N	%	n	%			
Ductal	22	67	9	27	2	6	33	0.80	0.20
Lobular	7	68	1	6	1	6	9	0.83	0.17
Invasive	4	50	4	50	0	0	8	0.75	0.25

AA vs AT:OR (95% CI) – 0.34(0.037 to 3.26)
– 1.57 (0.12 to 20.05)
AT vs TT: OR(95%CI)-0.50(0.03-6.86)
-0.42(0.02-10.74)
AA vs TT:OR (95% CI) OR (95% CI) – 2.44 (0.49 to 11.96)
– 1.00 (0.04 to 24.54)
Yates p value : 0.840;d.f-2;



Inference: The heterozygous AT genotype frequency of ATM -5144A>T gene polymorphism was elevated in invasive type of breast cancer.

Table2.1. Genotype distribution of CHEK2 1100delC polymorphism among cases and controls

	No: of individuals (%)			
CHEK2 C>G	**Controls (N=50)**	**Breast cancer (N=50)**	**OR (95% CI)**	**χ2 p**
aCo-dominant model				
C/C	33(66.0%)	26(52.0%)	**1.00(Ref)**	0.41
C/del	16(32.0%)	22(44.0%)	1.74(0.76-3.97)	
del/del	0(0.0%)	2(9.0%)	6.32(0.29-13.37)	
[b] Dominant model				
C/C	33(66.0%)	26(52.0%)	**1.00(Ref)**	0.12

C/del+del/del	16(32.0%)	24(48.0%)	1.90(0.84-4.30)	
[c] Recessive model				
CC+C/del	49(98.0%)	48(96.0%)	**1.00(Ref)**	0.48
del/del	0(0.0%)	2(4.0%)	5.10(0.23-10.06)	
[d] Over dominant model				
C/C+del/del	33(66.0%)	28(56.0%)	**1.00(Ref)**	0.24
C/del	16(34.0%)	22(44.0%)	1.62(0.71-3.67)	
Allele				
C	82(83.7%)	74(74.0%)	**1.00(Ref)**	**0.09**
del	16(16.3%)	26(26.0%)	**1.80(0.89-3.61)**	
HWE (p)	**1.87**	**1.03**		
*p<0.05; #p<0.10(χ2 p values)				

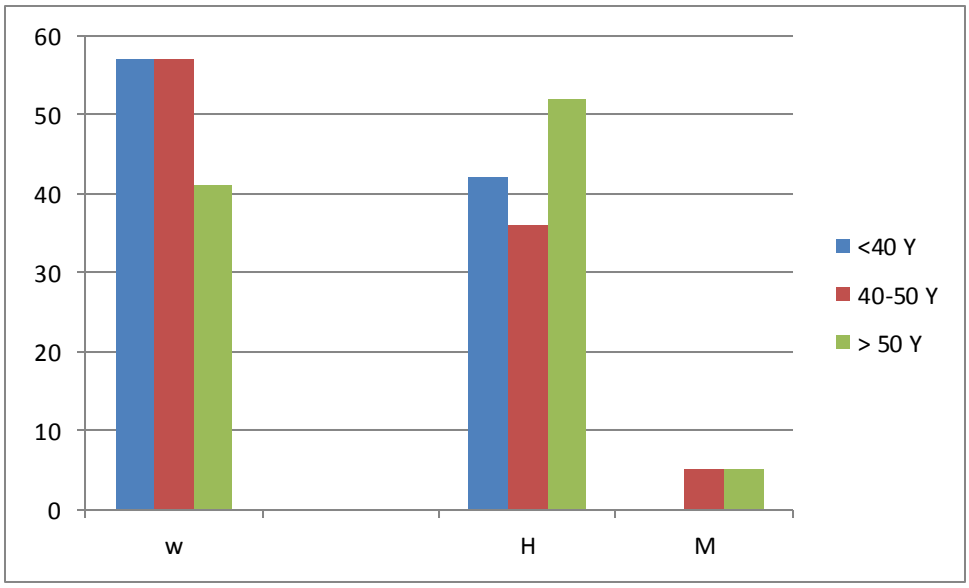
Inference: Heterozygous C/del genotype and variant del/del genotype frequencies of CHEK2 1100 delC polymorphism were elevated in breast cancer patients under co-domaint, dominant, recessive as well as over dominant model and there was border line significance of del allele frequency which revealed elevated risk for breast cancer development.

Table2.2. Genotype distribution of CHEK2 1100 del C>G polymorphism with respect to age at onset

Age	Genotype frequencies							Allelic Frequencies	
	C/C		C/del		del/del		Total	C	Del
	n	%	N	%	N	%			
< 40 years	8	57	6	42	0	0	14	0.79	0.21

International Journal of Research
Available at https://edupediapublications.org/journals
p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 03 Issue 11
July 2016

40-50 years	11	57	7	36	1	5	19	0.76	0.24
>50 years	7	41	9	52	1	5	17	0.77	0.33
C/C Vs C/del: OR (95% CI) –0.848 (0.20 to 3.51) –2.21 (0.08 to 61.40) C/C Vs del/del:OR (95% CI) –1.7143(0.40 to 7.29) –3.40 (0.11 to 96.70) C/del vs del/del :OR (95%CI) -2.60(0.08-75.49) -2.05(0.07-58.65) Yates p value : 0.958;d.f-2;									

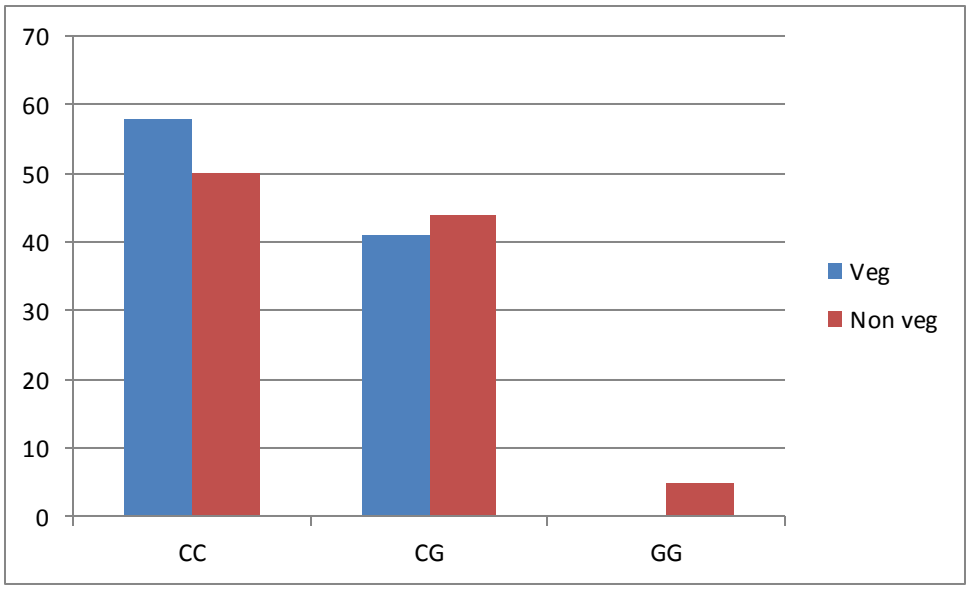


Inference: The heterozygous C/del genotype frequency of CHEK2 1100 del C gene polymorphism was elevated in the age group of >50 years among breast cancer cases.

Table2.3. Genotype distribution of CHEK2 1100delC polymorphism with respect to Diet

Diet	Genotype frequencies							Allelic Frequencies	
	C/C		C/del		del/del		Total	C	Del
	n	%	N	%	n	%			

Veg	7	58	5	41	0	0	12	0.79	0.21
Non veg	19	50	17	44	2	5	38	0.72	0.28
C/C vs C/del: OR (95% CI) –1.25(0.33-4.69 C/C vs del/del: OR (95% CI) –1.92(0.08-44.92) C/delvs del/del: OR (95%CI) -1.57(0.06-37.93) Yates p value : 0.98;d.f-2									

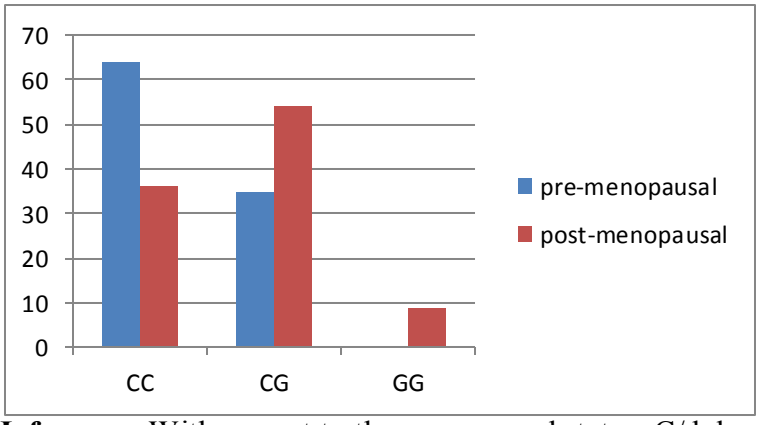


Inference: No significant association was observed for the CHEK2 1100del C polymorphism with respective to diet of breast cancer.

Table2.4. Genotype distribution of CHEK2 1100delC polymorphism with respect to Menopausal status

Menopausal status	Genotype frequencies							Allelic Frequencies	
	C/C		C/del		del/del		Total	C	Del
	n	%	N	%	n	%			
Pre-menopausal	18	64	10	35	0	0	28	0.82	0.18
Post- menopausal	8	36	12	54	2	9	22	0.64	0.36

C/C vs C/del :OR (95% CI) –2.70(0.82-8.80)
C/C vs del/del:OR (95% CI) –10.88(0.46-25.22)
C/del vs del/del: OR(95%CI)-4.20(0.18-97-55)
Yates p value :0.06; d.f-2



Inference: With respect to the menopausal status, C/del genotype was increased (54.0%) among Post- menopausal breast cancer patients. It was observed that T allele frequency of CHEK2 1100 delC polymorphism was also elevated in Post-menopausal cases compared to pre-menopausal patients.

Table2.5. Genotype distribution of CHEK2 1100del C polymorphism with respect to Lactation

Lactation	Genotype frequencies							Allelic Frequencies	
	C/C		C/del		del/del		Total	C	Del
	n	%	N	%	n	%			
Yes	21	55	15	39	2	5	38	0.75	0.25
No	5	41	7	58	0	0	12	0.71	0.29
C/C vs C/del:OR (95% CI) –1.96(0.52-7.37) C/C vs del/del:OR (95% CI) –0.78(0.03-18.75) C/del vs del/del : OR(95%CI)-0.41(0.02-9.73) Yates p value : 0.78; d.f-2									

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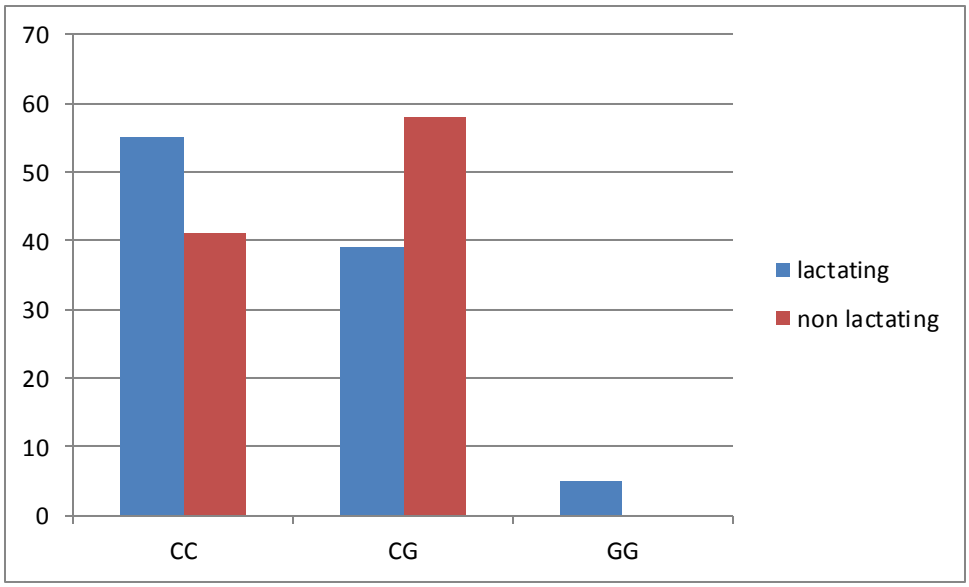
p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 03 Issue 11
July 2016



Inference:

C/del genotype frequency of CHEK2 1100delC polymorphism is elevated in Non-breastfeeding patients compared to breast feeding patients.

Table2.6. Genotype distribution of CHEK2 1100del C polymorphism with respect to Consanguinity

Consanguinity	Genotype frequencies							Allelic Frequencies	
	C/C		C/del		del/del		Total	C	Del
	n	%	N	%	n	%			
Consanguineous	10	63	5	31	1	6	16	0.78	0.22
Non consanguinous	16	47	17	50	1	3	34	0.72	0.28
CC vs C/del:OR (95% CI) –2.12(0.59-7.58) CC vs del/del:OR (95% CI) –0.62(0.03-11.15) C/del vs del/del : OR (95%CI)-0.29(0.01-5.59) Yates p value : 0.67;d.f-2									

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p-ISSN: 2348-6848
e-ISSN: 2348-795X
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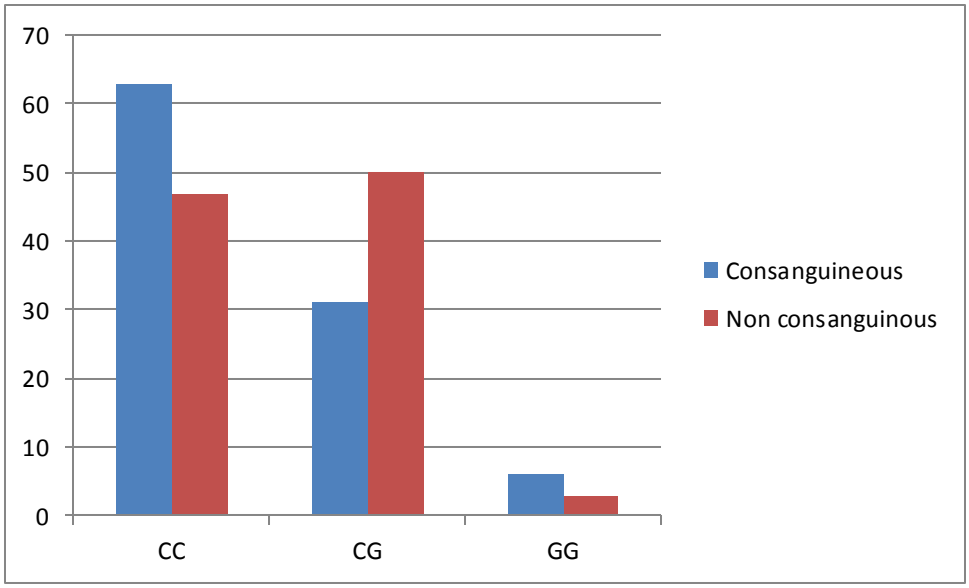
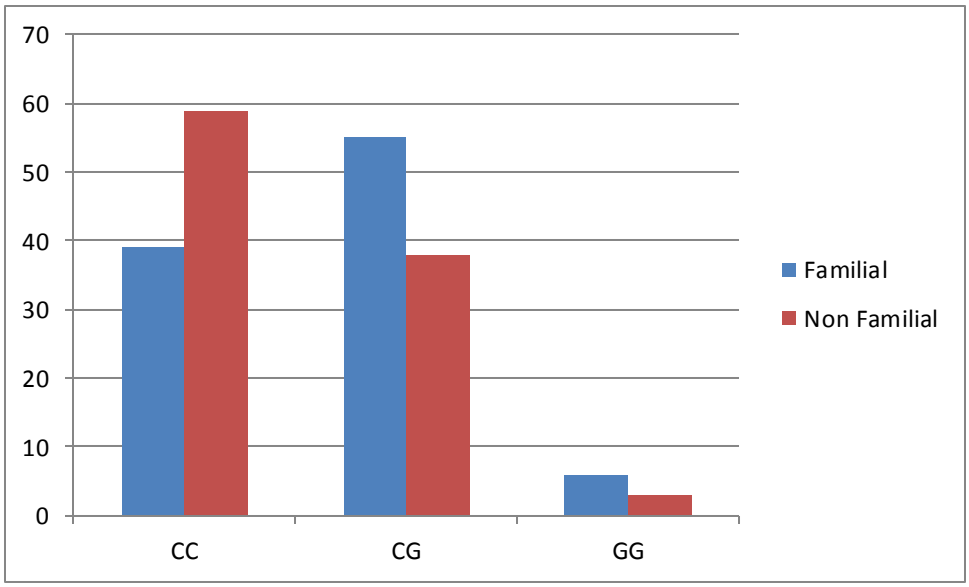


Inference: With respect to Consanguinity, it was observed that del allele frequency of CHEK2 1100delC polymorphism was slightly elevated in Non-consanguineous patients compared to consanguineous patients.

Table2.7. Genotype distribution of CHEK2 1100 del C polymorphism with respect to Familial incidence

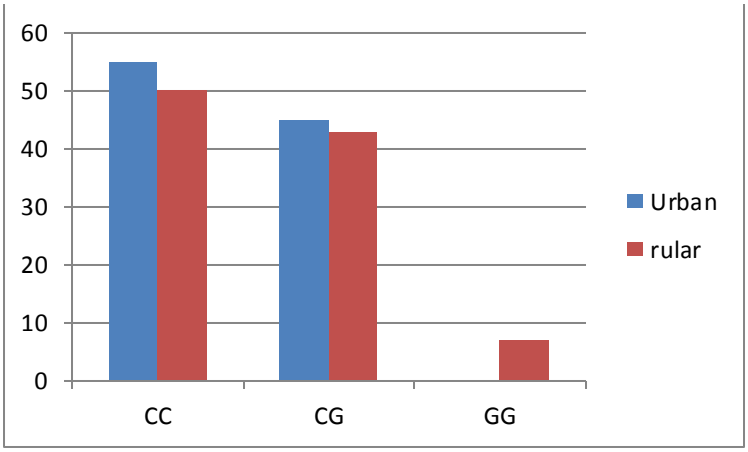
Familial incidence	Genotype frequencies							Allelic Frequencies	
	C/C		C/del		del/del		Total	C	Del
	n	%	N	%	n	%			
Familial	7	39	10	55	1	6	18	0.67	0.33
Non Familial	19	59	12	38	1	3	32	0.78	0.22
C/C vs C/del:OR (95% CI) –0.44(0.13-1.47) C/C vs del/del:OR (95% CI) –0.36(0.02-6.72) C/del vs del/del : OR(95%CI)-0.83(0.04-15.08) Yates p value : 0.55;d.f-2									



Inference: With respect to Familial incidence, the heterozygote C/del genotype (55.0%) and del allele (33.0%) frequencies are elevated among familial breast cancer cases.

Table2.8. Genotype distribution of CHEK2 polymorphism with respect to Area of living

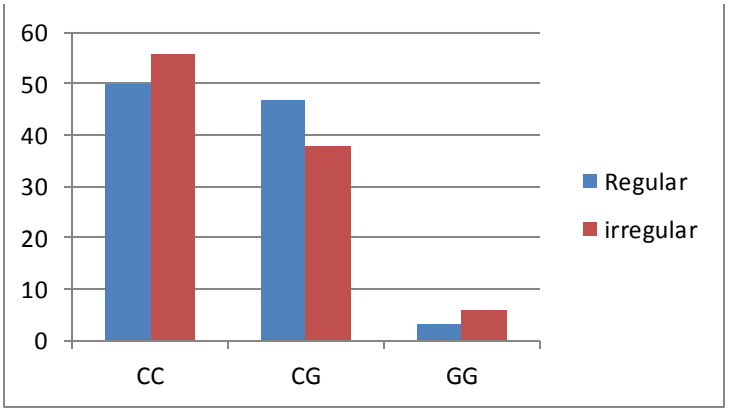
Area of living	Genotype frequencies							Allelic Frequencies	
	C/C		C/del		del/del		Total	C	Del
	n	%	N	%	n	%			
Urban	11	55	9	45	0	0	20	0.78	0.22
Rural	15	50	13	43	2	7	30	0.72	0.28



Inference: No significant association was observed for CHEK2 1100delC gene polymorphism with respective to area of living in breast cancer.

Table2.9. Genotype distribution of CHEK2 1100del C polymorphism with respect to menstrual cycle

Menstrual cycle	Genotype frequencies							Allelic Frequencies	
	C/C		C/del		del/del		Total	C	Del
	n	%	N	%	n	%			
Regular	17	50	16	47	1	3	34	0.74	0.26
Irregular	9	56	6	38	1	6	16	0.75	0.25
C/C vs C/del:OR (95% CI) –0.70(0.20-2.44) C/C vs del/del:OR (95% CI) –1.88(0.10-33.89) C/del vs del/del :OR(95%CI)-2.66(0.14-49.75) Yates p value : 0.94;d.f-2									



Inference: No significant association was observed for the polymorphism with respect to the history of menstruation.

Table2.10. Genotype distribution of CHEK2 1100delC polymorphism with respect to Estrogen receptor

Estrogen receptor	Genotype frequencies							Allelic Frequencies	
	C/C		C/del		del/del		Total	C	Del
	n	%	N	%	n	%			
Positive	12	48	12	48	1	4	25	0.72	0.28
Negative	14	56	10	40	1	4	25	0.76	0.24
C/C vs C/del: OR (95% CI) –0.71(0.22-2.23) C/C vs del/del :OR (95% CI) –0.85(0.04-15.22) C/del vs del/del :OR(95%CI)-1.20(0.06-21.72) Yates p value : 0.74;d.f-2									

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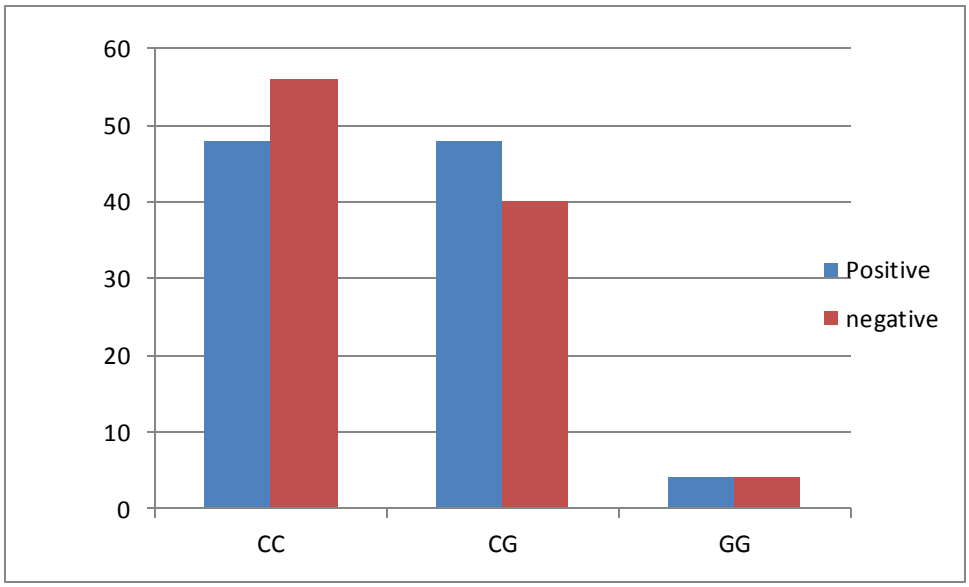
p-ISSN: 2348-6848
e-ISSN: 2348-795X
Volume 03 Issue 11
July 2016

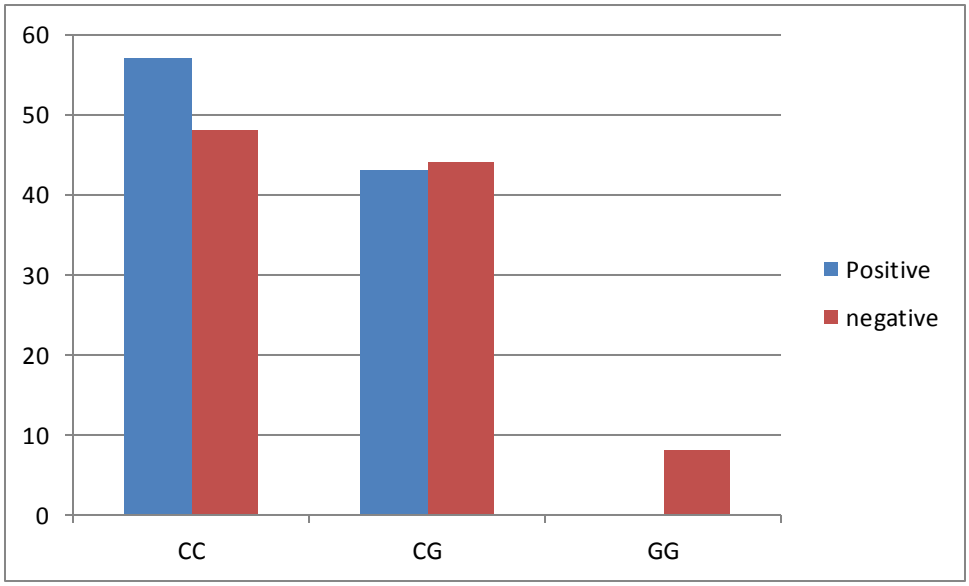


Inference: No significant association was observed between CHEK2 1100delC polymorphism and Estrogen receptor status.

Table2.11. Genotype distribution of CHEK2 1100delC polymorphism with respect to progesterone receptor

Progesterone receptor	Genotype frequencies							Allelic Frequencies	
	C/C		C/del		del/del		Total	C	Del
	n	%	N	%	n	%			
Positive	13	57	10	43	0	0	23	0.78	0.22
Negative	13	48	12	44	2	8	27	0.70	0.30

C/C vs C/del :OR (95% CI) –1.20(0.38-3.74)
C/C vs del/del :OR (95% CI) –5.00(0.21-11.42)
C/del vs del/del:OR(95%CI)-4.20(0.18-97.55)
Yates p value : 0.80;d.f-2;

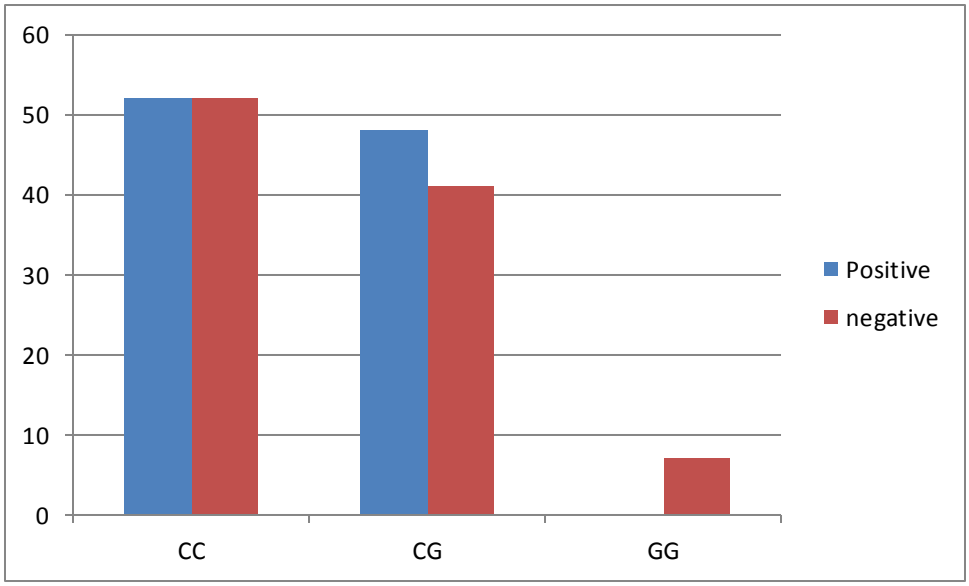


Inference: No significant association was observed between CHEK2 1100 del C polymorphism and Progesterone receptor status.

Table2.12. Genotype distribution of CHEK2 1100 del C polymorphism with respect to HER2 status

HER 2 status	Genotype frequencies							Allelic Frequencies	
	C/C		C/del		del/del		Total	C	Del
	n	%	N	%	n	%			
Positive	11	52	10	48	0	0	21	0.76	0.24
Negative	15	52	12	41	2	7	29	0.72	0.28

C/C vs C/del :OR (95% CI) –0.88(0.28-2.76)
C/C vs del/del :OR (95% CI) –3.70(0.16-84.92)
C/del vs del/del : OR(95%CI)-4.20(0.18-97.55)
Yates p value : 0.87;d.f-2;

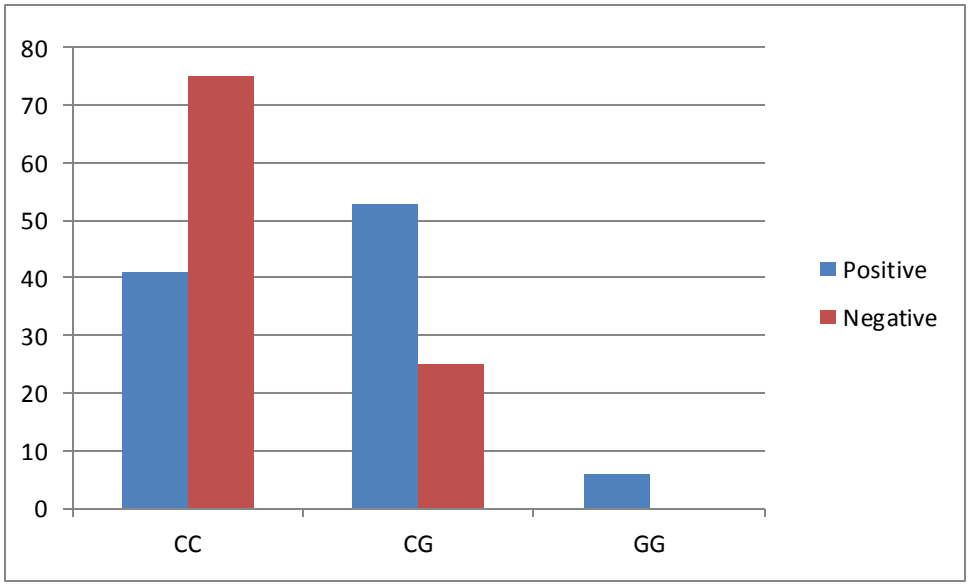


Inference: No significant association was observed for the CHEK2 1100 del C polymorphism with respect to HER2 receptor status.

Table2.13. Genotype distribution of CHEK2 1100delC polymorphism with respect to Axillary Node

Axillary Node	Genotype frequencies							Allelic Frequencies	
	C/C		C/del		del/del		Total	C	Del
	n	%	N	%	n	%			
Positive	14	41	18	53	2	6	34	0.68	0.32
Negative	12	75	4	25	0	0	16	0.88	0.12

C/C vs C/del:OR (95% CI) –0.25(0.06-0.98)
C/C vs del/del :OR (95% CI) –0.23(0.01-5.30)
C/del vs del/del :OR(95%CI)-0.82(0.03-20.30)
Yates p value :0.07;d.f-2

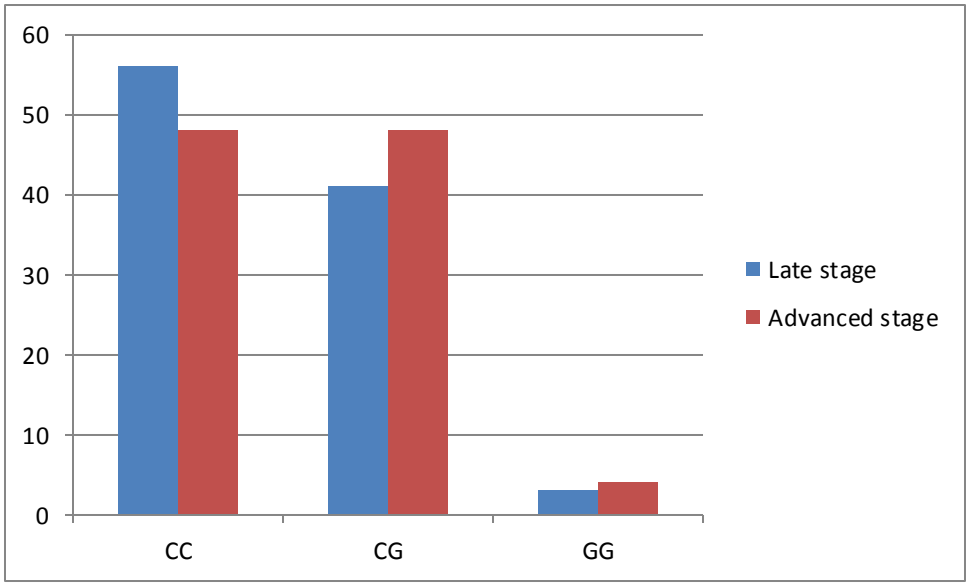


Inference: The C/del genotype frequency was elevated among patients who are axillary lymph node positive (53%) as compared to the cases who are negative for the axillary lymph node (25%).

Table2.14. Genotype distribution of CHEK2 1100 del C polymorphism with respect to tumor stage

Tumor stages	Genotype frequencies							Allelic Frequencies	
	C/C		C/del		del/del		Total	C	Del
	n	%	N	%	n	%			
Early stage	15	56	11	41	1	3	27	0.76	0.24
Advanced stage	11	48	11	48	1	4	23	0.71	0.29

C/C vs C/del:OR (95% CI) –1.36(0.43-4.27)
C/C vs del/del:OR (95% CI) –1.36(0.07-24.26)
C/del vs del/del:OR(95%CI)-1.00(0.05-18.08)
Yates p value : 0.81;d.f-2

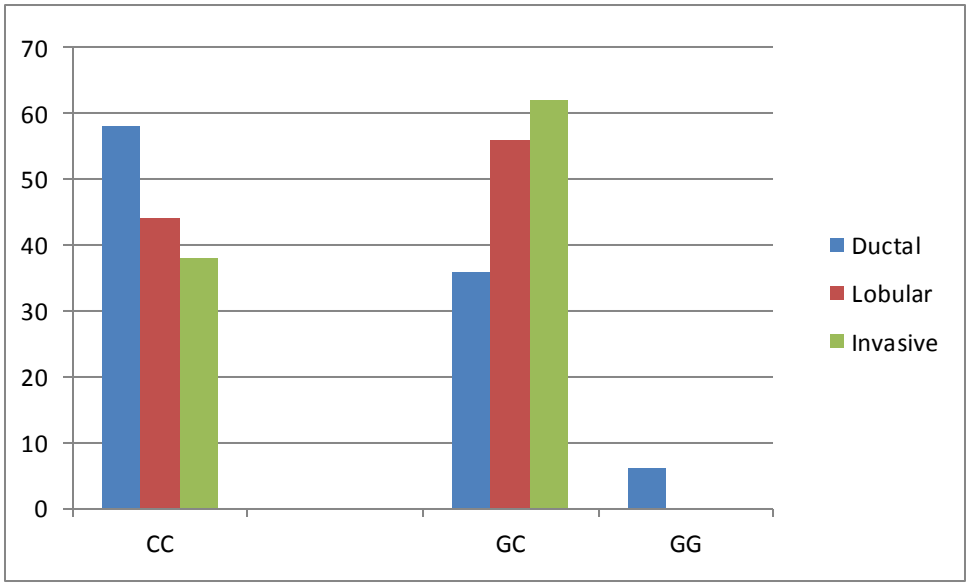


Inference: No significant association was observed between CHEK2 1100del C polymorphism and tumor stage.

Table2.15. Genotype distribution of CHEK2 1100 delC polymorphism with respect to Type of breast cancer

Type	Genotype frequencies							Allelic Frequencies	
	C/C		C/del		del/del		Total	C	Del
	n	%	N	%	n	%			
Ductal	19	58	12	36	2	6	33	0.76	0.24
Lobular	4	44	5	56	0	0	9	0.72	0.28
Invasive	3	38	5	62	0	0	8	0.69	0.31

C/C vs C/del:OR (95% CI) –1.98(0.44-8.87)
–0.86(0.03-21.36)
C/C vs del/del:OR (95% CI) –2.63(0.53-13.11)
–1.11(0.04-28.52)
C/del vs del/del: OR (95%CI)-1.00(0.05-18.08)
-1.00(0.06-18.09
Yates p value : 0.90;d.f-2;



Inference: The heterozygous C/del genotype frequency of CHEK2 1100 del C gene polymorphism was elevated in invasive type of breast cancer.

CONCLUSIONS

AT and TT genotype frequencies of ATMP -5144 A>T polymorphism were elevated in breast cancer patients under co-dominant, dominant, recessive as well as over dominant model and revealed increased risk for breast cancer development though not significant. Moreover, the AT genotype and T allele frequencies were elevated among the patients from rural area, with irregular menstrual cycles, with ER negative status and invasive type of cancer indicating their role in breast cancer progression. The genotype and allele frequencies of CHEK2 delC polymorphism are not deviated among controls and breast cancer patients. However, C/del and del/del genotype frequencies are associated with epidemiological variables like age, menopausal status, lactation, consanguinity, familial risk, menstrual cycle and clinical variables like advanced tumor stage, ER, HER2 receptor status. This might be due to deletion of C allele in kinase domain of CHEK2 gene which might have resulted in lowered expression/ decreased

function of CHEK2 gene thereby contributed in the progression of breast cancer. Hence, our results suggested that AT genotype of ATM -5144A>T polymorphism and C/del, del/del genotypes of CHEK2 delC polymorphism might be involved in the progression of breast cancer.

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