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Influence of Apolipoprotein E Gene Polymorphism on the Risk for Breast Cancer

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ABSTRACT Apolipoprotein E (APO E) is a polymorphic gene involved in lipid metabolism with three common alleles ε2, ε3 and ε4.The ε4 allele has been associated with elevated levels of cholesterol as well as greater risk for coronary heart disease and Alzheimer's disease. In the present study 110 cases of breast cancer and control were studied for APOE genotype distribution using PCR-RFLP (Polymerase chain reaction-Restriction fragment length polymorphism) technique. Significant association of APOE 3/4 with breast cancer (17.3%) was observed. Higher frequency of Breast cancer patients with steroid hormone receptor positive status (18%) were found to be of 3/4 genotype. The elevation in 3/4 genotype frequencies was also found in premenopausal group (21.6%) and in patients with advanced tumor (77.7%). Body mass index (BMI) and familial incidence did not show association with APOE genotype. The results suggest the influence of APOE genotype on development of breast cancer.

INTRODUCTION

Apolipoprotein E (apo E protein, APOE gene) is one of the key regulatory proteins in cholesterol and phospholipid metabolism. It plays an important role in binding of lipoprotein particles to the low density lipoprotein (LDL) receptor and the apo E receptor. (Poirier et al. 1993; Davignon et al. 1988; Mahley 1988).

The human APOE gene, located on chromosome 19q13.2, comprises of 3.7 kb DNA including 4 exons and 3 introns and encodes a polypeptide of 299 aminoacids (Das et al. 1985; Peik et al. 1989). Three common alleles of apo E isoforms E2, E3 and E4 as well as several other rare variants have been identified (Mahley 1988). E3 is the most common of these isoforms, and is distinguished by cysteine at position 112 (112 cys) and arginine at position 158 (158 arg) in the receptor-binding region of apo E (Weisgraber et al. 1981). The E4 isoform (112 arg and 158 arg) is associated with increased levels of total cholesterol and beta lipoprotein thereby increasing the susceptibility to heart disease. The E2 isoform (112 cys 158 cys) has reduced affinity to cellular receptors.

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The APOE ε4 allele, carried by approximately a third of the population, is a major genetic risk factor for Alzheimer's disease (Corden et al. 1993; Saunders et al. 1993; Striffmatter et al. 1993). It is associated with poor outcome following traumatic brain injury and intra cerebral hemorrhage (Alberts et al. 1995; Teasdall et al.1977; Mc Carron et al. 1998) and influences the plasma lipid profile and atherosclerosis (Uterrmann 1987). APOE also acts as an antioxidant, preventing the oxidation of LDL in the vasculature (Neely and Montine 2002). APOE is involved in smooth muscle cell proliferation and differentiation. It also modulates the immune response, which further affects tumorogenesis. APOE is highly expressed in many tumors, including brain, mammary and lung tumors. Recent studies have reported an association between APOE genotype and neoplastic disease as it is a potential inhibitor of cell proliferation and also plays significant role in antioxidant activity thereby inhibiting tumor growth and proliferation (Neimi et al. 2002).

The presence of different isoforms was reported to affect the tumor growth and proliferation at different levels. APOE ε2 allele was found to have largest protective effect, APOE ε4 the least and APOE ε3 has moderate (Miyata and Smith 1996). In a series of 35 prostatic carcinomas, there was an increased frequency of homozygosity for ε4 allele as compared to controls and possession of ε4 allele

was associated with earlier onset of the disease (Leher 1998). There was evidence that the APOE gene polymorphism influences the susceptibility to adenomas and carcinomas of the proximal colon, with relative protection of patients with APOE ε4 (Kervinen et al. 1996). The patients with primary brain tumors carrying APOE ε4 present at an older age and had a relatively good prognosis (Zunarelli et al. 2000). Some studies had not reported significant difference in ε4 allele frequencies between disease and control groups (Neimi et al. 2000; Iihan Yaylim et al. 2003). APOE ε4 allele had been related to breast carcinoma, that is women with one or two copies of the ε4 allele and high serum triglicerides had four times risk of developing breast carcinoma when compared with those having low triglyceride levels (Moysich et al. 1999). The APOE ε4 allele was found to influence the risk for breast cancer and was correlated with absence of HER2/neu status. The association of breast cancer risk with APOE ε2 and APOE ε4 was not observed among Caucasians and European women which suggest that influence of APOE polymorphism on breast cancer risk might vary across different populations (Nai-Wen Chang et al. 2005).

Hence, we have examined a series of breast carcinomas as well as controls to determine whether APOE polymorphism influences the risk for development of breast cancer in Indian population. Attempt was also made to understand the risk of APOE genotype in relation to other clinical variables such as BMI, familial incidences, onset of menopause, steroid hormone receptor status, and stage of the disease.

MATERIALS AND METHODS

A group of 110 breast cancer patients including 4 male breast cancer cases were selected for study by visiting the hospital on the days where outpatient clinic was run. Age matched healthy women without family history of breast cancer or any other cancers were selected to serve as the control group. Subjects were chosen from Nizam's Institute of Medical Sciences after confirmed diagnosis. The diagnosis of breast cancer was established by pathological examinations, mammography, FNAC, immunohistochemistry, etc. Detailed medical history and clinical information such as steroid hormone receptor status, stage of the disease was provided by the oncologist of the hospital. All the patients were interviewed personally for collection of data on epidemiological information such as age at onset and reproductive history. BMI was calculated as ratio of height in meters square and weight in Kg.

APOE Genotyping: Five milliliters of blood was collected in an EDTA vaccutainer from patients as well as controls. Genomic DNA was isolated using rapid non-enzymatic method (Nuremberg and Lahari 1991) and was used for amplification of exon 4 of APOE gene by PCR using specific oligonucleotide primers (James et al. 1990). Each amplification reaction contained 1μg of genomic DNA, 1pmol/μl of each primer, 10% dimethyl sulphoxide, 0.025U/μl of Taq polymerase, and 100mM dNTPs in a final volume of 30 μl. Each reaction mixture was heated at 95^0 C for 5min for initial denaturation and subjected to 30 cycles of amplification which includes denaturation at 95^0C for 1min, annealing at 60^0C for 1min, and extension at 70^0C for 2 min. PCR products were digested with 5U of HhaI (New England Biolabs) at 37^0C for overnight and run on 14% PAGE for 2.30 h. After electrophoresis, the gel was treated with eithidium bromide (5μg/μl) for 10min and DNA fragments were visualized on UV transilluminator. The size of bands was estimated using 100 base pair ladder on which genotyping was based viz:

ε3/3 91, 48, 35; **ε2/3** 91, 83, 48, 35; **ε3/4** 91, 72, 48, 35; **ε4/4** 72, 48, 35 and **ε2/4** 91, 83, 72, 48, 35 bands.(Fig :1)

Statistical Analysis*:* The results were analyzed using appropriate statistical tests. Odds ratio was estimated to calculate the relative risk for each genotype to develop disease. Differences in genotype frequency distribution between disease and control groups was done using 2x2 contingency χ^2 and χ^2 test for heterogeneity.

RESULTS

One hundred and ten breast cancer patients and healthy controls were analysed for APOE genotype distribution .The mean age at diagnosis of breast cancer in the present sample was 47.6 years. 36% of cases had familial history for cancer (breast cancer or other cancer). The APO E genotype distribution was studied with respect to clinical variables such as menopausal status, body mass index, steroid hormone receptor status (estrogen receptor and progesterone receptor status) and familial incidence. The APOE genotype association was also studied in relation to staging of the tumor.

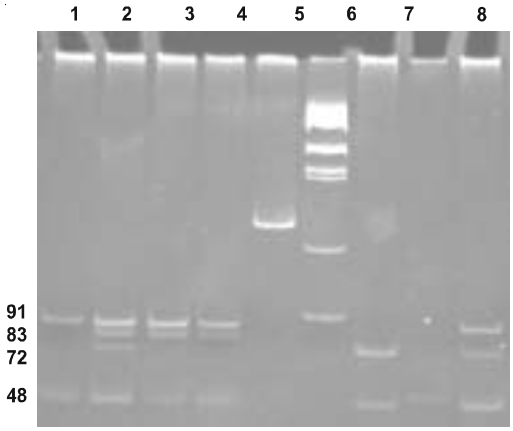

Fig.1. Apolipoprotein E genotypes

Lanes:
1. ε3/3 91, 48 bp
2. ε2/4 91, 83, 72, 48 bp
3&4. ε2/3 91, 83, 48 bp
5. Uncut 244 bp
6. 100 bp ladder
7. ε4/4 72, 48 bp
8. ε3/4 91, 72, 48 bp

Table 1 shows frequency distribution of APOE genotype in both breast cancer and controls. In general, APOE 3/3 was found to be the most frequent genotype in disease and controls. However there was an elevation of APOE 3/4 genotype frequency in disease (17.3%) as compared to control group (5.5%) with corresponding increase in ε4 allele frequency. Both disease and control groups did not deviate from Hardy Weinberg equilibrium. The premenopausal breast cancer women also showed considerable increase in APOE 3/4 genotype frequency (21.6%) as compared to postmenopausal women (13.6%). The ε4 allele frequency was also elevated in premeopausal group as compared to postmenopausal group (Table 2). When the data was analysed with respect to estrogen receptor status and progesterone receptor status, there was an increase in frequency of APOE 3/4 genotype among breast cancer cases with steroid hormone receptor positive status (Table 3).

Among 18 patients with APOE 3/4 genotype, 14 (77.7%) of them had advanced tumor (III &IV), and 4 patients (22.2%) had early tumor (I &II). However 57.9% of patients with E3/3 genotype (40 out of 69) had early tumor and the remaining (42%) had advanced tumor. The allele frequency was also elevated in patients with advanced tumor (0.16) as compared to the frequency in patients with early tumor (Table 4).

There was no significant association of APOE genotype with familial incidences and body mass index (Table 5, 6).

Table 1: APOE genotypes and allele frequencies in disease and controls

	Genotypes										*Allele Frequency*		
	3/3		*3/4*		*4/4*		*2/4*		*2/3*		*ε2*	*ε3*	*ε4*
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*			
D	84	76.7	19	17.3	1	0.9	0	0	6	5.5	0.03	0.88	0.09
C	93	84.5	6	5.5	0	0	2	1.8	9	8.2	0.05	0.91	0.04
Total	177		25		1		2		15				

Odds ratio: 3/3 Vs 3/4 = 0.28 (CI 95%= 0.1088-0.7479)
2/3 Vs 3/4 =0.21 (CI 95%=0.0529-0.8383)
Hardy Weinberg equilibrium: Breast Cancer χ^2 = 0.1762(p=0.9813)
Controls χ^2 = 6.173(p=0.1035)

Table 2: APOE genotypes and allele frequencies in pre menopausal and postmenopausal women

	Genotypes										*Allele Frequency*		
	3/3		*3/4*		*4/4*		*2/4*		*2/3*		*ε2*	*ε3*	*ε4*
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*			
Pre	39	76.5	11	21.6	1	1.96	0	0	0	0	0.0	0.87	0.13
Post	45	76.3	8	13.6	0	0	0	0	6	10.2	0.05	0.88	0.07
Total	84		19		1		0		6				

Odds ratio: 3/3 Vs 3/4 = 0.63 (CI 95%=0.2303-1.7247)
1. χ^2 = 0.82 (p=0.3652)

Table 3: APOE genotypes and allele frequencies in steroid hormone receptor status

	Genotypes										*Allele Frequency*		
	3/3		*3/4*		*4/4*		*2/4*		*2/3*		*ε2*	*ε3*	*ε4*
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*			
+	38	76	9	18	0	0	0	0	3	6	0.03	0.88	0.09
-	27	84	3	9	1	3	0	0	1	3	0.01	0.91	0.08
Total	65		12		1		0		4				

Odds ratio: 3/3 vs. 3/4 = 0.46 (CI 95%=0.1161-1.896)
1. χ^2 = 0.573 (p=0.45)

Table 4: APOE genotypes in stage of the disease

Stage	*Genotypes*									
	3/3		*3/4*		*4/4*		*2/4*		*2/3*	
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*
I	3	75	1	25	0	0	0	0	0	0
II	37	84.1	3	6.8	0	0	0	0	4	9.0
III	22	68.7	9	28.1	0	0	0	0	1	3.1
IV	7	58.3	5	41.7	0	0	0	0	0	0
Total	69		18		0		0		5	

Odds ratio: 3/3 and 3/4 = 4.83 (CI 95%=1.44-16.184)
2. χ^2 = 9.28 (p=0.0097)

Table 5: APOE genotypes and allele frequencies in familial incidences

	Genotypes										*Allele Frequency*		
	3/3		*3/4*		*4/4*		*2/4*		*2/3*		*ε2*	*ε3*	*ε4*
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*			
F	27	79.4	6	17.6	0	0	0	0	1	2.9	0.01	0.89	0.09
NF	55	75.3	12	16.4	1	1	0	0	5	7	0.03	0.87	0.09
Total	82		18		1		0		6				

Odds ratio: 3/3 Vs 3/4 = 0.9818 (CI 95% =0.3325-2.8993)
1. χ^2 = 0.001 (p=0.97)

Table 6: APOE genotypes in body mass index

BMI	*Genotypes*									
	3/3		*3/4*		*4/4*		*2/4*		*2/3*	
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*
<20	2	50	2	50	0	0	0	0	0	0
20-22.6	17	89.5	2	11	0	0	0	0	0	0
22.6-30	19	73.1	4	15.4	0	0	0	0	3	11.5
30	14	93.3	1	6.7	0	0	0	0	0	0
Total	52		9		0		0		3	

1. χ^2 = 5.128 (p=0.16)
Footnotes:
1. 2x2 contingency χ^2 3/3 Vs3/4
2. χ^2 for heterogeneity (p<0.05)

DISCUSSION

The present study attempted to evaluate the role of APOE polymorphism in development of breast cancer. The mean age at onset of breast cancer in the present sample was found to be 47.6 years, which is an agreement with the previous study (Takatani et al. 1991). The average age of female breast cancer patients in Taiwan was found to be 47.4 years, which was lower than that reported in American Caucasian women (58.0). 18.18% of breast cancer patients were

carriers of ε4 allele where as only 7.27% of controls carried ε4 allele. The association of ε4 allele with breast cancer was found to be 11.9% in previous report (Moysich et al. 2000).

An increased frequency of ε4 allele has been reported in prostrate carcinoma. Patients homozygous for ε4 had early age at onset, which might be due to decreased antioxidant activity of apo E ε4 (Leher 1998).

APO E 3/4 genotype frequency was elevated among premenopausal breast cancer cases (21.6%) as compared to post menopausal (13.6%) indicating that 3/4 genotype might confer risk to develop breast cancer at an earlier age. Such comparison could not carried out in control cases due to small sample size of 3/4 genotype in control data. The frequency of APO E 3/4 genotype was higher among patients with steroid hormone receptor positive status suggesting the role of estrogen as a potent inducer of mammary cell proliferation. It has been demonstrated that estrogen activates estrogen receptor and results in transcription of various genes that are involved in cell proliferation (Emi et al. 2002).

APOE 3/4 genotype showed significant elevation among patients with advanced stage of the disease. APOE coordinates transport of lipids systemically via the blood stream and is responsible for the delivery of cholesterol and phospholipids to cell by receptor mediated uptake. There are evidences that this lipid delivery system varies in efficiency according to APOE genotype because of variation in the affinity of the different apoE isoforms to the cell surface receptors. It has been postulated that apoE provides tumor with the lipid substrate required for tumor growth and that if the lipid uptake system is a rate limiting step then tumor may grow at different rates in patients with different APOE genotypes (Rubinsztein et al. 1994). The decreased antioxidant activity of APOE ε4 might predispose to the development of cancer.

In conclusion, our data suggested that APOE genotype influences the susceptibility to develop breast cancer.

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