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*Report*

# Influences of apolipoprotein E polymorphism on the risk for breast cancer and HER2/neu status in Taiwan

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**Summary**

Apolipoprotein E (APOE) polymorphism plays an important role in lipid metabolism. Preliminary evidence suggests that APOE genotype appears to be a risk factor for not only cardiovascular disease, but also Alzheimer's disease and cancer. We screened the APOE genotype in 290 breast cancer patients and 232 non-cancer controls and determined the relationship between APOE gene polymorphism and breast cancer in Taiwan. We found risk for breast cancer was associated with the APOE genotype ($\chi^2 = 8.652$, $p = 0.013$). Carriers of the ε4 allele were more common in breast cancer cases than carriers of ε3 allele ($p = 0.004$, OR = 1.786, 95% CI: 1.197–2.664). In addition, the ε4 allele is also associated with HER2/neu negative status in breast cancer patients ($p = 0.006$, OR = 0.277, 95% CI: 0.111–0.693). No significant associations between APOE genotype and tumor grade, TN classification, progesterone receptor, estrogen receptor, lymphatic invasion, or recurrence of breast cancer were in evidence. These results suggest that the APOE ε4 allele may be a risk factor for breast cancer and correlates with HER2/neu negative status.

## Introduction

Apolipoprotein E (Apo E) is a 299-amino acid glycoprotein which consists of four exons and three introns spanning 3597 nucleotides located on the long arm of chromosome 19 [1]. Apo E is a normal constituent of very low-density lipoprotein (VLDL) and high-density lipoprotein (HDL) and has an important role in lipid metabolism by serving as a ligand to hepatic lipoprotein receptors [1–3]. In humans there are three functionally distinct isoforms of the protein (E2, E3, and E4), encoded by the corresponding alleles ε2, ε3, and ε4 [1–3]. These isoforms differ in amino acid sequence at positions 112 and 158. The ApoE2 isoform differs from the wild-type ApoE3 isoform by a single amino acid change resulting in minimal receptor binding activity and reduced clearance of chylomicron remnants, while the ApoE4 isoform results in faster chylomicron clearance [4].

APOE alleles combine to generate six possible genotypes ranking from most to least common: 3/3, 4/3, 3/2, 4/4, 4/2, and 2/2 [1–3]. The ε4 allele is associated with increased risk for coronary heart disease [2, 5], Alzheimer's disease [6–8] and prostatic carcinomas [9]. In contrast, the ε4 allele is associated with decreased risk for colon cancer [10].

Recently, the ε4 allele has found to be linked to breast cancer. Among Caucasian American women possessing one or two copies of APOE4 allele, those with high concentrations of triglycerides had four times the risk of developing breast cancer when compared with women with low triglyceride concentrations [11]. However, compared with women in possession of the APOE3 allele, there were no associations with breast cancer risk for Caucasian [12] or English [13] women with either the APOE2 or APOE4 alleles. Taken together, the impact of APOE polymorphism on breast cancer risk may vary across diverse populations.

In population studies, APOE allele frequencies were found to differ in 11 populations studied thus far [14, 15]. The major APOE allele in all populations is ε3 (frequency range from 39.8% in Sudanese to 72.1% in Japanese). Northern Europeans (Finns, Germans) tend to have higher frequencies (14–19%) of the ε4 allele than southern Europeans (French, Italians; 7–12%). Nigerians, Japanese, and Finns have relatively low frequencies (3–4%) of ε2. Therefore, to determine whether APOE genotypes (ε2, ε3, and ε4) are related to the risk of

breast cancer in Taiwan, the relationship between APOE polymorphism and clinically diagnosed breast cancer was studied.

## Materials and methods

### *Study population*

The study population is composed of female Taiwanese ranging in age from 25 to 73 years. Breast cancer patients ($n = 290$; mean 47.41 ± 10.12 SD) were recruited from the China Medical University Hospital and Feng Yuan Hospital. The informed consent was obtained from the subjects. It is designed to embrace a number of the clinical measures and outcomes of breast carcinoma including menopause status, family history of breast cancer, laterality, pathology, primary tumor size, TNM classification, tumor grade, estrogen receptor status, progesterone receptor status, HER2/neu status, positive lymph node number, vascular invasion, lymphatic invasion, distant metastasis, and recurrence of breast cancer. Non-cancer controls ($n = 232$; mean 40.20 ± 10.68 SD) were recruited from the Taichung Blood Center and China Medical University Hospital; and infection of HBV (Hepatitis B virus), HCV (Hepatitis C virus), HIV (Human immuno-deficiency Virus), and HTLV (Human T-lymphotropic virus) were excluded. Tumor stage group was determined according to the AJCC/UICC TNM Classification and Stage grouping [16]. Histological grade was determined according to the modified Bloom-Richardson grade [17]. The esterogen receptor, progesterone receptor, and HER2/neu status were determined by immunohistochemical staining methods [18, 19].

### *Polymorphism analysis*

Genomic DNA was extracted from whole blood by using the Viogene® isolation kit (Viogene, Taiwan). APOE genotyping was performed according to the method of Hixon and Vernier [20]. Exon 4 of APOE gene was amplified by polymerase chain reaction (PCR). The amplicon was generated using the following PCR primers: forward primer (5′-ACAGAATTCGCCCCGGCCTGG TACAC-3′); and reverse primer (5′-TAAGCTTGGCA CGGCTGT-CCAAGGA-3′). Then, the PCR product was digested by the *HhaI* restriction enzyme. Digests were resolved by electrophoresis through a 4.5% agarose gel, run at 100 V for 40 min, stained with ethidium bromide and visualized by UV transillumination.

### *Statistical analysis*

Statistical analysis was performed using SPSS software (version 10.0.7C). Difference in age was analyzed by two-sample *t*-test. APOE genotypes and allele frequencies of breast cancer patients and non-cancer controls were compared by using Pearson $\chi^2$-test and Fisher's exact test. Logistic regression analysis tested the associations of APOE polymorphism with the risk for breast cancer and with clinical outcome measures.

## Results

We studied a total of 522 individuals: 290 breast cancer patients and 232 non-cancer controls. There was no significant difference between the breast cancer patients and non-cancer controls with respect to age ($p = 0.277$).

The APOE genotype distribution and allele frequencies are shown in Table 1. APOE polymorphism was classified as ε2 carrier (2/2, 2/3, or 2/4), ε3 carrier (3/3), or ε4 carrier (3/4 or 4/4). The ε3 allele frequency occurred at a lower rate (70.7%) in the 290 breast cancer cases as compared to the 232 non-cancer controls (75.2%). A significant difference in the APOE genotypes between breast cancer patients and non-cancer controls was observed ($\chi^2 = 8.652$, df = 2, $p = 0.013$). Logistic regression analysis revealed a significant influence of the ε4 allele on the risk for breast cancer ($p = 0.004$, OR = 1.786, 95% CI: 1.197–2.664, ε4 versus ε3).

Presence or absence of HER2/neu was associated with APOE polymorphism in breast cancer patients ($\chi^2 = 10.307$, df = 2, $p = 0.006$, Table 2). The results of a logistic regression analysis with patients classified in the HER2/neu negative status indicated carries of the ε4 allele were significantly enriched in this group ($p = 0.006$, OR = 0.277, 95% CI: 0.111–0.693, ε4 versus ε3).

There was no significant difference between APOE polymorphism and the following clinical outcomes:

*Table 1.* Correlation of APOE genotypes and allele frequencies in the study population

| Allele/Genotype | | Patients | Controls |
|---|---|---|---|
| | | Frequency, % | |
| *Allele* | | | |
| ε2 | | 9.6 | 9.7 |
| ε3 | | 70.7 | 75.2 |
| ε4 | | 19.6 | 15.1 |
| | | *n* (%) | |
| *Genotype* | | | |
| ε2 carrier | 2/2 | 11 (3.8) | 14 (6.0) |
| | 2/3 | 24 (8.3) | 4 (1.7) |
| | 2/4 | 10 (3.4) | 13 (5.6) |
| ε3 carrier | 3/3 | 145 (50.0) | 145 (62.5) |
| ε4 carrier | 3/4 | 96 (33.1) | 55 (23.7) |
| | 4/4 | 4 (1.4) | 1 (0.4) |
| APOE | $\chi^2$ | df | *p* |
| versus study populations | 8.652 | 2 | 0.013 |

*Table 2*. Association between APOE genotypes and HER2/neu status, estrogen receptor status, progesterone receptor status, and lymphatic invasion in breast cancer patients

| | APOE genotype[a], *n* (%) | | | | APOE versus status | | |
|---|---|---|---|---|---|---|---|
| | Total | ε2 | ε3 | ε4 | $\chi^2$ | df | *p* |
| *HER2/neu* | | | | | | | |
| Positive | 51 | 11 (21.6) | 33 (64.7) | 7 (13.7) | 10.307 | 2 | 0.006 |
| Negative | 102 | 12 (11.8) | 51 (50.0) | 39 (38.2) | | | |
| *ER*[b] | | | | | | | |
| Positive | 120 | 19 (15.8) | 61 (50.8) | 40 (33.3) | 0.004 | 2 | 0.998 |
| Negative | 82 | 13 (15.9) | 42 (51.2) | 27 (32.9) | | | |
| *PR*[c] | | | | | | | |
| Positive 96 | 96 | 17 (17.7) | 44 (45.8) | 35 (36.5) | 2.545 | 2 | 0.280 |
| Negative | 96 | 14 (14.6) | 55 (57.3) | 27 (28.1) | | | |
| *Lymphatic invasion* | | | | | | | |
| Positive | 93 | 18 (19.4) | 46 (49.5) | 29 (31.2) | 0.948 | 2 | 0.623 |
| Negative | 175 | 26 (14.9) | 89 (50.9) | 60 (34.3) | | | |

[a]APOE genotype was classified as ε2 carrier (2/2, 2/3, 2/4), ε3 carrier (3/3), and ε4 carrier (3/4, 4/4).
[b]Estrogen receptor.
[c]Progesterone receptor.

progesterone receptor, estrogen receptor, lymphatic invasion (Table 2), tumor grade, and TN classification (Table 3).

## Discussion

This study evaluated the association between the risk for breast cancer and APOE gene polymorphism. Epidemiological studies have revealed that age–incidence curves of breast cancer in Asians differs from those in Caucasians [21]. The peak of age distributions for East Asian women occurs in the range from 40 to 50 years, contrasting with the incidence occurring at greater than 50 years of age in Western women. In our study, the genotype distribution of APOE polymorphism in our Taiwanese study population agrees with the findings of

*Table 3*. Correlation of APOE genotypes with TN classification or tumor grade in breast cancer patients

| | APOE genotype[a], *n* (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| | Total | ε2 | ε3 | ε4 | $\chi^2$ | df | *p* |
| | | | | | APOE versus T classification | | |
| *T classification*[b] | | | | | | | |
| 1 | 94 | 12 (12.8) | 45 (47.9) | 37 (39.4) | 5.002 | 4 | 0.287 |
| 2 | 150 | 29 (19.3) | 70 (46.7) | 51 (34.0) | | | |
| 3 | 21 | 2 (9.5) | 14 (66.7) | 5 (23.8) | | | |
| | | | | | APOE versus N classification | | |
| *N classification*[c] | | | | | | | |
| 0 | 187 | 27 (14.4) | 95 (50.8) | 65 (34.8) | 0.860 | 2 | 0.650 |
| 1 or 2 | 79 | 17 (18.7) | 43 (47.3) | 31 (34.1) | | | |
| | | | | | APOE versus Tumor grade | | |
| *Tumor grade* | | | | | | | |
| I | 31 | 5 (16.1) | 16 (51.6) | 10 (32.3) | 0.406 | 4 | 0.982 |
| II | 119 | 21 (17.6) | 57 (47.9) | 41 (34.5) | | | |
| III | 65 | 11 (16.9) | 34 (52.3) | 20 (30.8) | | | |

[a]APOE genotype was classified as ε2 carrier (2/2, 2/3, 2/4), ε3 carrier (3/3), and ε4 carrier (3/4, 4/4).
[b]T1 classification: tumor 2 cm or less in greatest dimension. T2 classification: tumor more than 2 cm but not more than 5 cm in greatest dimension. T3 classification: tumor more than 5 cm in greatest dimension.
[c]N0 classification: no regional lymph node metastases. N1 classification: metastasis to movable ipsilateral axillary lymph nodes. N2 classification: metastases to ipsilateral axillary nodes fixed to one another or to other structures.

Eichner et al. [15] who estimated APOE genotype frequencies from diverse populations for the purposes of international and ethnic comparisons. The average age of female breast cancer patients in Taiwan (mean 47.4 ± 10.1 SD) is younger than those reported in American Caucasian women (mean 58.0 ± 9.8 SD) [12]. Carriers of the ε4 allele are at significantly increased risk of breast cancer (1.786-fold) as compared to carriers of the ε3 allele in Taiwan. The ε4 allele frequency in American Caucasian breast cancer patients was approximately 11.9% [12], whereas in Taiwanese breast cancer patients the ε4 allele frequency was is estimated at 19.6%. The higher ε4 allele frequency observed in Taiwanese women may represent a more important risk factor for earlier onset of breast cancer in the Taiwanese population. Moreover, this increased genetic risk may be shared more broadly in Asian populations.

Another possible mechanism explaining the apparent increased risk for breast cancer in carriers of the ε4 allele may be related to this allele's triglyceride-elevating effect. APOE4 reduces triglyceride clearance from plasma, resulting in persistently elevated triglyceride concentrations, which could result in decreased sex hormone-binding globulin levels and elevated levels of free estradiol [22]. It has been demonstrated that estrogen activates estrogen receptor and results in transcription of various genes that are involved in cellular proliferation. In particular, the presence of estrogen receptor and/or progesterone receptor is an important diagnostic feature in breast cancer development and progress [23, 24]. However, we did not find any correlation between APOE polymorphism and estrogen receptor status or progesterone receptor status.

Furthermore, we found patients possessing ε4 alleles were associated with HER2/neu-negative status. Human HER2/neu gene overexpression is associated with a faster rate of tumor growth and an increased rate of metastasis [25]. HER2/neu-positive patients tend to have a poor prognosis and a decreased disease-free survival and overall survival time [25,26]. Accordingly, we tested for a synergistic effect between HER2/neu status and the APOE ε4 allele and did not find a significant influence on the recurrence of breast cancer and the distant metastasis (data not shown). Failure to demonstrate an association between APOE ε2 or ε4 carrier status and a measure of tumor progression, the cell proliferation index (MIB-1) [13], highlights the need of consistent measures and independent replication studies.

In conclusion, our findings suggest that APOE polymorphism plays an important role in the development of breast cancer. The APOE ε4 allele influences the risk for breast cancer and is correlated with absence of HER2/neu status. Taken together, the APOE genotype should be explored further to evaluate its potential as a marker for not only cardiovascular and Alzheimer's disease risk, but for breast cancer risk.

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