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SHORT REPORT

# Apolipoprotein E Allelic Frequency Altered in Women with Early-onset Breast Cancer

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**Abstract:** Among women, the most prevalent type of cancer is breast cancer, affecting 1 out of every 8 women in the United States; in Puerto Rico, 70 out of every 100,000 will develop some type of breast cancer. Therefore, a better understand of the potential risk factors for breast cancer could lead to the development of early detection tools. A gene that has been proposed as a risk factor in several populations around the world is Apolipoprotein E (apoE). ApoE functions as a mechanism of transport for lipoproteins and cholesterol throughout the body, with 3 main isoforms present in humans (apoE2, apoE3, and apoE4). Whether or not apoE4 is a risk factor for breast cancer remains controversial. Previous studies have either included test subjects of all ages (20–80) or have focused on late-onset (after age 50) breast cancer; none has concentrated specifically on early-onset (aged 50 and younger) breast cancer. The objectives of this study was to examine (in a Puerto Rican population) the differences in the relative frequency of occurrence of apoE4 in non-breast cancer versus breast cancer patients and to examine, as well, the potential differences of same in early- versus late-onset patients. We found an increased frequency of apoE4 (odds ratio 2.15) only in early-onset breast cancer survivors, which is similar to the findings of those studies that combined or adjusted for age as well as for an association between apoE4 and decreased tumor size. ApoE is also a potential risk factor for long-term cognitive effects after chemotherapy and affects response to hormone replacement. Our data supports the theory that knowing the apoE genotype of women who are at risk of developing breast cancer may be beneficial, as such knowledge would aid in the prediction of tumor size and the development of treatment regimens.

**Keywords:** breast cancer, apoe, puerto rican, risk factor, early-onset

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## Cancer Surveillance Report

Accounting for 20% of all malignancies in women (globally), breast carcinoma (BC) is the most common cancer to affect this population.[1–3] The estimated annual incidence of BC worldwide is about one million cases.[2,3] Studies conducted by the American Cancer Society indicate that there are 2.5 million breast cancer survivors in the United States (2005), which number is expected to increase by 31% by 2015.[2] This means that 1 out of every 8 women is expected to have some form of breast cancer in her lifetime.

Extensive research has been conducted in the last 10 years regarding potential genetic risk factors, one of those genes being apolipoprotein E (apoE).[4–8] The apoE gene is responsible for the metabolism of lipoproteins and cholesterol as well as for the distribution of these (metabolized) substances throughout the body.[9] There are 3 main alleles found in humans, apoE2, apoE3, and apoE4. Changes in apoE4 allelic frequency are normally examined in association with the increased risk of age-related cognitive impairments and the development of Alzheimer's disease (AD), particularly in females.[6–8] African Americans and Caribbean and Mexican Hispanics also display similar risk factors associated with the apoE4 allele, such as lowering the age of onset of sporadic AD and reducing memory performance in individuals 65 years and older.[6–8] However, changes in apoE4 allelic frequency are not limited to those with neuronal conditions.

Various investigations also indicate that apoE4 allelic frequency is altered in various types of cancer including breast cancer and early onset prostate cancer.[6–8] The potential risk in the development of breast cancer and odds ratios appears to depend on the populations examined. In women from Turkey and Finland, either with or without breast cancer, no differences appear in apoE4 allelic frequency.[6–8] In contrast, there are indications of an increase in apoE4 allelic frequency in women from Taiwan, Italy, and Africa.[6–8] One common limitation in most of the studies that hypothesize that apoE may be a potential risk factor in the development of breast cancer is that they did not include subjects who developed breast cancer at or before the age of 50.[6–8] Studies that included young subjects with early onset of breast cancer grouped all patients together or adjusted for age (with ages ranging from the 20s to the 80s).[6–8] Those studies that used broad age ranges were more likely to report an association between being an apoE4 carrier and having a risk for breast cancer.[6–8] Therefore, an explanation for the discrepancies that appear in the association of apoE4 and breast cancer risk is the inclusion of early-onset breast cancer patients. Understanding the genetics of early-onset breast cancer would increase the probability of early detection of tumors before the need for invasive treatments.

As of 2003, the incidence of women with breast cancer in Puerto Rico was 78 per 100,000, with 50.4 women per 100,000 receiving invasive treatment including surgery and/or chemotherapy.[10–12] The mortality rate in 2003 was 16 per 100,000, resulting in approximately 1,540 deaths that year, making it the most prevalent, most commonly diagnosed cancer and the highest cause of cancer-related death among Puerto Rican women.[12] The overall incidence of breast cancer is similar to levels reported in the United States (across all ages).[12] However, the probability of Puerto Ricans developing the more advanced stage-4 cancers is 3.6 times higher, and Puerto Rican women have a 1.6 times greater risk of mortality compared to Hispanic whites.[1] These rates are similar to other Hispanic white populations including Mexicans.[1] Breast Cancer is the most common form of cancer for Puerto Rican women, both for those residing on the island and for those in the mainland United States.[13]

In regards to genetics, Puerto Ricans and other Caribbean Hispanics are very diverse and include Hispanic (European and South American), Caucasian, and African genes, along with other native groups. Studies involving the haplogroups of 800 mitochondrial DNAs (both randomly and systematically selected) suggest that the Puerto Rican population is genetically mixed, composed primarily of African, European and Amerindian genes.[14,15] This population represents individuals affected by multiple ancestral genetic pools with implications that genetic risk factors are not specific to one ethnic group. The purpose of this study was to examine whether apoE allelic frequency was altered in Puerto Rican women and whether there were differences in the frequency with which this allele appeared in women with early-onset as compared to those with late-onset breast cancer. Spreadsheets containing the obtained data were converted and analyzed using SPSS 15.0 software. Statistical differences

between groups were determined by ANOVA, the presence of apoE (apoE4 carrier vs. non-apoE4 carrier) and/or individuals diagnosed with breast cancer as the between-participant factors, followed by Tukey-Kramer post-hoc tests when appropriate. A Student's *t*-test was used to analyze differences between non-breast cancer and breast cancer subjects separated by age group (21–50, 51–89).

The collection of samples used in this study was approved by the IRB of the Ponce School of Medicine, Ponce, PR, and participating hospitals for use in a large scale case-control study for which one of the co-authors (J.L.M.) is the PI. An informed consent was administered to each participant (cases and controls) for interviewing, drawing blood samples and, for cases, obtaining tumor material and pathology reports. The design for this case-control study called for utilizing incident cases; it began with recently diagnosed, histopathologically confirmed breast carcinoma cases. These recently diagnosed cases had not received chemotherapy and/or radiotherapy. These cases were recruited primarily through clinicians in the cities of Ponce, San Juan, and Yauco as well as other selected collaborating cities throughout Puerto Rico representing approximately 58 out of the 78 municipalities (counties) on the island. Recruitment sites included the Ponce School of Medicine Outpatient Clinic, Auxilio Mutuo Hospital (San Juan), Damas Hospital (Ponce) and St. Luke's Hospital (Ponce). Participants (cases and controls) were all of Hispanic origin. An epidemiological questionnaire soliciting information and variables that were related to breast carcinoma risk was provided to each participant. Only cases with primary and metastatic breast carcinoma tumors (rather than secondary or other types of cancer) were studied. The pathology report from each patient was obtained in order to learn the tumor grade, tumor size, and other clinically relevant information.

Controls were women who had never been diagnosed with breast cancer and who were recruited consecutively from individuals visiting gynecological and primary-care medical offices in Puerto Rico for their routine mammography and other types of screening. There was a possibility that controls would have more weight than cancer cases; that potential confounding factor as well as any other confounding factors that became apparent were carefully analyzed and the properly adjustments made.[16] Controls and cases were recruited from a population whose members all visited the same sites (clinics, physicians' offices, hospitals); any control who later developed breast cancer would be treated at the site from which she was recruited. Two main criteria determined the eligibility of controls: 1. any potential participant had to have had a normal clinical breast exam by her primary physician in the six months prior to enrollment, and 2. she had to have had a normal mammogram. These criteria reduced the likelihood of the existence of breast carcinoma among controls.

Approximately thirty milliliters of peripheral blood was obtained from each participant and stored in heparinized tubes. The lymphocytes were then isolated by the Ficoll gradient technique and suspended in freezing media containing 10% dimethyl sulfoxide, 40% RPMI-1640 medium, 50% fetal bovine serum, and 1% antibiotic/antimycotic the resulting solution was divided into 2.0 ml aliquots and stored in a freezer at –80 °C. These lymphocytes were later thawed in batches for DNA isolation. All of the samples came from Puerto Rican women, both with (205) and without (229) breast cancer, ranging in age from 22 to 80 years old. Only those with available DNA as of August 2009 were used for this study. There was no overall difference or differences among ≤50 and 51≥ subjects with respect to age, family history of breast cancer, body mass index, whether they ever had been pregnant, or number of live births among age-matched groups (Table 1). This lack of association between family history of breast cancer and age of onset has also been seen in other Hispanic populations.[17] Civil status was the same among age-matched controls, though the older women were more likely to be widows (Table 1).

ApoE genotyping was performed by polymerase chain reaction with the BioRad DNA Engine Thermal Cycler from the Molecular Biology Core Laboratory. Approximately 200 ng of purified DNA was required for a high-quality product. Reactions of 25 µL were carried with a PCR Master Mix (Promega) Taq 50 U/mL, 400 µM of e/a dNTPs, and 3 mM $MgCl_2$. The primers used were: (F4) 5'-ACAGAATTCGCCCCGGCCTGGTACAC-3' (1 mM) and (F6) 5'-TAAGCTTGGCACGGCTGTCCAAGG-3' (1 mM).[18] Reactions were denatured at 95 °C for 5 min, followed by 35 cycles of primer annealing at 60 °C for 1 min, extension at 70 °C for 2 min, and

**Table 1.** Demographics of non-breast cancer and breast cancer patients.

| | Non-breast cancer (22–50 yrs) | Breast cancer (22–50 yrs) | *P*-value | Non-breast cancer (51–89 yrs) | Breast cancer (51–89 yrs) | *P*-value |
|---|---|---|---|---|---|---|
| Age ± S.E.M. (n) | 41.7 ± 0.6 (106) | 43.0 ± 0.6 (63) | 0.38 | 61.8 ± 0.8 (123) | 63.8 ± 0.7 (142) | 0.06 |
| % Family history of breast cancer (n) | 49.0 ± 0.1 (98) | 42.6 ± 0.1 (61) | 0.51 | 40.3 ± 0.05 (119) | 34.5 ± 0.04 (139) | 0.36 |
| Body mass index (n) | 26.5 ± 0.5 (104) | 26.6 ± 0.6 (63) | 0.89 | 27.9 ± 0.4 (123) | 27.8 ± 0.5 (142) | 0.86 |
| % Ever been pregnant (n) | 22.6 ± 0.05 (104) | 25.4 ± 0.05 (63) | 0.68 | 17.9 ± 0.03 (123) | 18.3 ± 0.03 (142) | 0.92 |
| # Live births (n) | 2.0 ± 0.1 (91) | 2.3 ± 0.2 (55) | 0.36 | 3.1 ± 0.2 (110) | 2.8 ± 0.1 (125) | 0.20 |
| % Smoke (n) | 11.6 ± 0.03 (103) | 14.2 ± 0.04 (63) | 0.63 | 12.5 ± 0.03 (120) | 15.6 ± 0.03 (141) | 0.47 |
| % Alcohol (n) | 37.6 ± 0.2 (93) | 45.7 ± 0.2 (56) | 0.79 | 43.7 ± 0.2 (112) | 15.6 ± 0.03 (134) | 0.25 |
| Civil status (n) | 105 | 63 | | 122 | 142 | |
| Married | 67.4% | 68.5% | | 65.5% | 44.9% | |
| Single | 22.0% | 13.7% | | 13.8% | 25.7% | |
| Divorced | 9.6% | 13.7% | | 17.2% | 16.2% | |
| Widow | 1.0% | 4.0% | | 3.4% | 13.2% | |

denaturation at 95 °C for 1 min. This process was followed by a final extension of 10 min. After 3-hours of digestion with CfoI/HhaI (Promega), products were run on 4% agarose gel, and bands were identified by size according to restriction fragment length polymorphism.[19]

The frequency of overall apoE4 carriers within this population was 21.0% for women without breast cancer and 24.4% in breast cancer patients. There was no significant difference in frequency for any of the genotypes when grouping those with early-onset and late-onset breast cancer ($p = 0.30$) and those similar to excepted frequencies based on Caucasian populations. However, with a $2 \times 2$ Analysis of Variance (ANOVA), when comparing subjects that were diagnosed at or before the age of 50 versus those that were diagnosed at or after age 51 with appropriate controls, we found a significant differences in genotype ($F = 5.43$, $p < 0.02$, Table 2). As seen in previous studies, in the late-onset breast cancer patients there were no differences in apoE allelic frequency.[4,5] In contrast, we found twice as many apoE3/apoE4 alleles in women who were 50 or younger, with a significant difference in those who are apoE carriers ($F = 4.00$, $p < 0.05$, Table 2), and no change in the frequency with which the apoE2/apoE3 genotype is found. The odds ratio for the frequency of apoE4 in women 50 and under between non-breast cancer and breast cancer patient's was 2.15 (CI: 1.05–4.39). The apoE4 frequencies found in our population of early-onset individuals are

**Table 2.** Frequency of apoE genotypes in non-breast cancer and breast cancer patients.

| | Non-breast cancer (21–50 yrs) | Breast cancer (21–50 yrs)[1,2] | Non-breast cancer (51–89 yrs) | Breast cancer (51–89 yrs) |
|---|---|---|---|---|
| n | 106 | 63 | 123 | 142 |
| apoE2/apoE2 | 0 | 0 | 0 | 0 |
| apoE2/apoE3 | 10.4% | 7.9% | 6.5% | 5.7% |
| apoE3/apoE3 | 74.0% | 58.7% | 70.7% | 74.6% |
| apoE3/apoE4 | 14.4% | 33.3% | 22.0% | 17.6% |
| apoE2/apoE4 | 1.9% | 0 | 0 | 0 |
| apoE4/apoE4 | 0.9% | 0 | 0.8% | 2.1% |
| Non-apoE4 carrier | 81.1% | 66.7% | 77.2% | 79.6% |
| apoE4 carrier | 18.9% | 33.3% | 22.8% | 20.4% |

[1]Significant difference in allelic frequency ($P < 0.02$).
[2]Significant difference in frequency of apoE4 carriers ($P < 0.05$).

the same as others found in an age-adjusted breast cancer population.[20] We also found that apoE4 was associated with decreased tumor size in younger women, but not with tumor grade (Table 3). As other studies have made 51 (as opposed to 50) the cut-off age for comparisons,[21] we repeated analysis and found the same significant effect (decreased tumor size) in early-onset breast cancer patients in terms of apoE genotype ($F = 4.76$, $p < 0.03$) and apoE4 allelic frequency ($F = 3.95$, $p < 0.05$). These data suggest that apoE4 may be a risk factor for the early onset of breast cancer and affect tumor size in Puerto Rican women who have breast cancer.

Particular genes like apoE also affect the response to chemotherapy and needed hormone treatment, which is an important factor to consider in the treatment of breast cancer. In Greek post-menopausal women, apoE4 carriers displayed reduced response to Tamoxifen.[22] Research suggests that knowing whether or not a patient's gene profile includes the apoE polymorphism is critical when pondering the risks and benefits of using estrogen in hormone therapy.[23,24] Studies on apoE as a potential genetic risk factor in breast cancer or lymphoma survivors found that even eight years after chemotherapy, apoE4 carriers displayed impairments specifically in visual memory, spatial ability, and psychomotor functioning.[25]

The current study suggests the importance of age of diagnosis as a factor in the relationship between apoE genotype and the risk for breast cancer. This study will need to be confirmed with additional ethnic/racial populations. Nonetheless, these findings suggest a strong association between apoE genotype and age of diagnosis of breast cancer in Puerto Rican women. There is a controversy concerning when women should start getting mammograms; such tests are currently recommended for women between 45 and 50 years of age, depending on case and family history. This might turn out to be too late for some women, considering the fact that the highest incidence rate for ethnic minorities is age 50 and below and that those with early onset are likely to have more aggressive disease.[26] Our data indicates that studies on genetic markers in early-onset breast cancer patients may encourage women to rethink when they should start getting mammograms or other types of screening procedures.[27,28]

Knowing an individuals apoE genotype would allow the physician more information on how a patient may response to hormones and chemotherapy if they are diagnosed. Taken together with past microarray analyses of single nucleotide polymorphisms in middle European white women,[23] our study supports the idea that breast cancer patients would benefit from health care professionals having access to their genetic profiles; such knowledge would aid in the design of treatment regimens as well as improve the effectiveness of genetic counseling.

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**Table 3.** Effects of apoE4 in breast cancer patients.

| | Non-apoE4 carrier (22–50 yrs) | apoE4 carrier (22–50 yrs) | *P*-value | Non-apoE4 carrier (51–89 yrs) | apoE4 carrier (51–89 yrs) | *P*-value |
|---|---|---|---|---|---|---|
| n | 30 | 18 | | 90 | 20 | |
| Grade 1 | 20.0% | 16.7% | | 15.6% | 20.0% | |
| Grade 2 | 46.7% | 44.4% | | 47.8% | 55.0% | |
| Grade 3 | 30.0% | 38.9% | | 31.1% | 25.0% | |
| Grade 4 | 3.3% | 0 | | 5.6% | 0 | |
| Total tumor grade | 2.2 ± 0.1 | 2.6 ± 0.4 | 0.80 | 61.8 ± 0.8 | 63.8 ± 0.7 | 0.06 |
| Tumor size | 2.5 + 0.4 | 1.2 + 0.3 | 0.02[1] | 2.2 + 0.2 | 1.8 + 0.4 | 0.36 |

[1]Significant difference in tumor size.

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## Disclosures

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

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