Apolipoprotein E genetic polymorphism, serum lipoprotein levels and breast cancer risk: A case-control study

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Abstract. The purpose of this study was to evaluate the association between apolipoprotein E (APOE) allelic frequency, serum lipoproteins and breast cancer (BC). We conducted a nested case-control study within a cohort including 47 cases and 165 controls. Polymerase chain reaction-restriction fragment length polymorphism analyses of the APOE polymorphism were performed. In general, participants with the genotype including alleles e2 and e3 tended to have lower serum triglycerides, total cholesterol and low-density lipoprotein cholesterol levels and higher high-density lipoprotein (HDL) cholesterol levels compared to participants homozygous for the e3 allele and participants heterozygous for the e3 and e4 alleles, respectively. BC patients exhibited higher mean levels of total serum cholesterol (P=0.070), dietary fat intake (P=0.020) and dietary cholesterol intake (P=0.017) compared to control subjects. The allelic distribution between the two groups revealed that the presence of the e2 allele was positively associated with the absence of BC, whereas the e4 allele was positively associated with the BC case group (P=0.019). The distribution of the APOE genotypes was not significantly different between cases and controls (P=0.172). The concomitant presence of the e2 and e4 alleles was positively associated with the absence of BC and e4/e4 homozygosity was positively associated with BC (P=0.021). Our findings suggested that APOE polymorphism plays an important role in the development of BC, particularly when associated with higher serum triglyceride levels.

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Introduction

Apolipoprotein E (APOE) is a member of the apolipoprotein gene family and plays an important role in lipid metabolism by mediating the binding of lipoprotein particles to the low-density lipoprotein (LDL) receptor and the APOE receptor (1). The genetic polymorphisms of APOE are among the most extensively investigated, particularly due to the effects of APOE on lipid profiles and the risk of coronary heart disease (2). The structural gene locus of APOE is polymorphic (3): there are three common alleles, namely e2, e3 and e4, coding for three isoforms, namely E2, E3 and E4, respectively, which produce three homozygous genotypes (E2/E2, E3/E3 and E4/E4) and three heterozygous genotypes (E2/E3, E2/E4 and E3/E4) (4). It was demonstrated that women with one or two copies of the e4 allele and those with high concentrations of triglycerides had four times the risk of developing breast cancer (BC) compared to women with low triglyceride levels. The increase in BC risk may be attributed to a variety of factors, such as the effect of reduced triglyceride clearance from the plasma, resulting in constantly elevated concentrations, which may result in decreased sex hormone-binding globulin levels (5). However, the evidence remains inconclusive, as other studies reported no association between the presence of either the e2 or the e4 allele and the rate of tumor cell proliferation or clinical outcome in Italian BC patients (6).

The APOE2 protein differs from the wild-type protein, APOE3, by a single amino acid change resulting in minimal receptor binding activity and reduced clearance of chylomicron remnants (7). APOE4 differs from APOE3 in that a different amino acid substitution results in faster chylomicron clearance (7). In general, compared to individuals with the e3 allele, the levels of total and LDL cholesterol tend to be

lower in those with the e2 allele and higher in those with the e4 allele. The e4 allele has been associated with increased risk of coronary heart disease (8) and Alzheimer's disease (9). By contrast, it was reported that there is a lower frequency of the e4 allele among patients with proximal tumors of the colon compared to that among healthy individuals (8).

Dietary fat intake has been hypothesized to be associated with BC risk based on animal studies (10), ecological studies (11) and studies on migrants from areas with low fat intake to those with high fat intake (12). An association between cholesterol intake and BC risk has not been established (13). The failure to identify an association between fat intake and BC in those studies may be due to the interindividual differences in fat consumption within populations being inadequately detected with epidemiological methods (14) and due to the measurement error that is inherent in dietary questionnaires (15). Furthermore, the assessment of the recent diet may be the wrong exposure and earlier dietary habits may be more important. In addition, the intercountry differences may be associated with other, correlated differences in dietary intake or other exposures.

Serum lipoprotein levels have also been investigated in relation to BC etiology as a potential mediating effect of dietary fat on BC risk and as an independent risk factor. The associations between serum or plasma total cholesterol, high-density lipoprotein (HDL) cholesterol, LDL cholesterol and triglycerides have been widely investigated; however, the results have been inconsistent. Certain studies reported no adverse effect of lipoproteins (16,17), whereas others reported an excess risk associated with elevated total cholesterol (18,19) and triglyceride levels (14,20,21) or inverse associations with total cholesterol (19) or HDL cholesterol levels (20,21).

The association between APOE and BC has not been definitively established and the results are often conflicting. Despite the availability of data associating the frequency of APOE genotypes with cancer in general, the number of studies investigating the association between BC and APOE is currently limited, whereas this association in the Brazilian population has yet to be investigated. In this context and since APOE is known to modify the association between dietary intake and blood lipid levels, we aimed to evaluate the association between APOE allelic frequency, serum lipoproteins and BC risk in a sample of women from Porto Alegre, Brazil.

Subjects and methods

Study design and participant recruitment. This was a nested case-control study within a cohort. Our sample included BC patients and heathy controls recruited from the Porto Alegre Breast Health Intervention Cohort. In April, 2004, a large population-based cohort study was initiated in Porto Alegre, the capital of the southern Brazilian state of Rio Grande do Sul. The cohort aimed to collect demographic, epidemiological and risk factor data from a large sample of females aged ≥15 years and test a model for community-based BC screening for women aged 40-69 years, as described elsewhere (22,23).

Considering that the average frequency of the e4 allele previously described in a sample of Taiwanese women without BC was 5.4% (24), with a power of 80%, a confidence interval (CI) set at 95% and a case:control ratio of 1:3, we estimated an ideal sample size of 52 cases and 156 controls. The case group included patients with carcinoma *in situ* or invasive tumor histologically confirmed.

Study variables. Demographic and clinical information screening data were obtained through chart review. The study variables included age, age at menarche, age at first pregnancy, period of exclusive breast feeding (months), number of children, menopausal status and medications used. The body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). The abdominal circumference (AC) was measured with a measuring tape at midway between the lower rib and the iliac crest.

Biochemical analyses were performed on venous blood obtained following a recommended 12-h overnight fasting. The samples were collected in tubes without anticoagulants for the quantification of lipids and glucose. For result analyses, the following normal values were considered: plasma total cholesterol <200 mg/dl, plasma triglycerides <150mg/dl; plasma HDL >50 mg/dl; plasma LDL-C <160 mg/dl (25).

Molecular analyses. Peripheral blood samples from all the patients were collected in EDTA tubes. DNA was extracted with the standard Illustra Blood genomicPrep Mini Spin kit (GE Healthcare, Piscataway, NJ, USA). APOE genotyping was performed by polymerase chain reaction (PCR)-restriction fragment length polymorphism as described by Hixson and Vernier (26). Briefly, the 244-bp amplicon obtained by PCR was enzyme-restricted using *Hha*I and resulted in fragments of different sizes, which were resolved by polyacrylamide gel electrophoresis as follows: i) fragments of 91 and 83 bp for genotype E2E2; ii) fragments of 91, 48 and 35 bp for genotype E3E3; and (iii) fragments of 72, 48 and 35 bp for genotype E4E4. Throughout the manuscript we collectively refer to the genotypes E3E3 as APOE3, E2E3 and E2E4 as APOE2 and E3E4 and E4E4 as APOE4.

Statistical analyses. SPSS software, version 18.0 (SPSS Inc., Chicago, IL, USA) was used for data handling and statistical analyses. To evaluate the differences in demographic, anthropometric, lifestyle, gynecological and obstetrical characteristics between the two groups, we used the Student's t-test for normally distributed variables, the Mann-Whitney test for non-parametric variables without a normal distribution and the Chi-square test for categorical variables. Logistic regression analysis was used to calculate the odds ratios (ORs) and 95% CIs for the APOE polymorphism. The ORs were adjusted by the dietary consumption of proteins, fats, carbohydrates and cholesterol. The variables of age, age at menarche and number of children were not included, as the P-values were not <0.20 following logistic regression analyses.

Ethics. This study was approved by the Institutional Research and Ethics Committees of the participating institutions (Clinics Hospital of Porto Alegre protocols: 05-182 and 08/070; Moinhos de Vento Hospital protocol 2008/86). All the participants provided written informed consent prior to their inclusion in the study.

Table I. Clinical and demographic characteristics of breast cancer patients and healthy controls.

	Cases (n=47)		Controls (n=165)		
Characteristics	No. (%)	Median (IIQ or SD)	No. (%)	Median (IIQ or SD)	P-value
Age (years)[a]		57.6 (10.6)		56.1 (8.1)	0.295
BMI (kg/m^2)[b]		28.2 (5.9)		28.9 (7.2)	0.206
Abdominal circumference (cm)[b]		93.0 (17.0)		94.0 (17.0)	0.850
Age at menarche (years)[b]		13.0 (3.0)		13.0 (2.0)	0.584
Age at first pregnancy (years)[b]		20.0 (5.0)		22.0 (8.0)	0.365
Exclusive breast feeding (months)[b]		15.0 (45.0)		12.0 (32.0)	0.526
No. of children, n (%)					
1-3	32 (68.1)		114 (53.77)		
>4	15 (31.9)		51 (46.2)		0.730
Menopausal status, n (%)					0.434
Yes	31 (67.4)		102 (61.1)		
Hormone therapy use, n (%)					0.179
Yes	5 (11.1)		33 (19.8)		
Contraceptive use, n (%)					0.403
Yes	34 (73.9)		133 (79.6)		

[a]Median and standard deviation (SD), t-test. [b]Median and interquartile range (IIQ), Mann-Whitney non-parametric test and Chi-square test. BMI, body mass index.

Results

Subjects. A total of 47 BC patients and 165 healthy controls were included in this study. The characteristics of the sample are summarized in Table I. There were no statistically significant differences between the groups regarding mean age, BMI, AC, age at menarche, age at first pregnancy, breastfeeding, number of children, menopausal status, hormone and contraceptive use.

APOE genotype distribution. The distribution of the APOE genotypes in the total sample and by disease status is shown in Table II. Regardless of the disease status, nearly all the participants (90.0%) carried at least one e3 allele, 34.5% carried an e4 allele and 17.5% carried an e2 allele, which was consistent with a recent study based on Caucasian women (27). Table II shows the mean serum lipoprotein levels by APOE genotype. In general, participants with the genotype including alleles e2 and e3 tended to have lower serum triglycerides, total cholesterol and LDL cholesterol levels and higher HDL cholesterol levels compared to participants homozygous for allele 3 and those heterozygous for alleles e3 and e4, respectively. This pattern was more apparent for the total sample and the BC cases and less apparent for the controls. Only one woman in the control group was homozygous for allele e4 and exhibited the highest serum levels of triglycerides, LDL and HDL.

Serum lipoproteins and dietary fat intake. There were differences between the cases and controls regarding the mean levels of total serum cholesterol (P=0.070), dietary fat intake (P=0.020) and dietary cholesterol intake (P=0.017). There were no significant differences in serum HDL, LDL and triglyceride levels between the groups, as shown in Table III.

Allele and genotype distribution in BC patients and controls. The analysis of the allelic distribution between the two groups (Table IV) revealed that the presence of the e2 allele was positively associated with the absence of BC, whereas the e4 allele was positively associated with the BC case group (P=0.019). The distribution of the APOE genotypes, however, exhibited no significant difference between the cases and the controls (P=0.172). Moreover, the concomitant presence of the e2 and e4 alleles was positively associated with the absence of BC and e4/e4 homozygosity was positively associated with the presence of BC (P=0.021), suggesting a dominant negative effect of the e2 over the e4 allele.

Discussion

To the best of our knowledge, this is the first study to investigate the association between the APOE genotype, serum lipoprotein levels and BC risk in Brazilian women. In the total sample, we observed that women who had at least one e4 allele presented with higher triglyceride levels compared to those with an e2 allele (Table II). Previous studies reported an increased risk of BC in association with elevated triglyceride levels (14,20,21) and the possibility of a number of biological mechanisms underlying this association has been investigated. The increase in the BC risk may be due to a variety of factors,

Table II. Mean serum lipoprotein levels by APOE genotype in breast cancer patients and healthy controls[a].

APOE genotype	No. (%)	Total cholesterol (mg/dl) Mean (SD)	HDL cholesterol (mg/dl) Mean (SD)	LDL cholesterol[b] (mg/dl) Mean (SD)	Triglycerides (mg/dl) Mean (SD)
Total sample (n=212)					
2/3	20 (9.4)	210.3 (47.9)	56.0 (21.1)	121.7 (44.9)	162.7 (103.3)
2/4	17 (8.1)	185.0 (32.7)	53.6 (11.2)	100.7 (31.3)	153.2 (83.1)
3/3	119 (56.1)	201.3 (45.8)	51.4 (14.6)	116.7 (44.8)	165.8 (84.0)
3/4	52 (24.5)	216.9 (52.4)	50.1 (13.8)	128.9 (53.2)	189.2 (155.2)
4/4	4 (1.9)	187.2 (22.1)	48.5 (11.1)	90.2 (56.3)	242.7 (123.9)
Cases (n=47)					
2/3	4 (8.5)	253.5 (72.5)	51.0 (14.1)	166.3 (56.4)	181.0 (159.3)
3/3	28 (59.6)	189.9 (55.2)	48.4 (12.1)	109.7 (51.4)	159.1 (61.8)
3/4	12 (25.5)	194.7 (50.3)	50.2 (15.2)	106.5 (56.4)	190.3 (241.1)
4/4	3 (6.4)	195.3 (18.4)	43.7 (6.6)	114.2 (35.9)	187.3 (67.9)
Controls (n=165)					
2/3	16 (9.7)	199.5 (35.2)	57.2 (22.7)	110.6 (35.4)	158.1 (91.3)
2/4	17 (10.3)	185.0 (32.6)	53.6 (11.1)	100.7 (31.3)	153.2 (83.1)
3/3	91 (55.2)	204.8 (42.3)	52.3 (15.2)	118.9 (42.6)	167.8 (89.9)
3/4	40 (24.2)	223.6 (51.8)	50.1 (13.6)	135.7 (50.9)	188.8 (122.8)
4/4	1 (0.6)	163	63	180.2	409.0

[a]In Control Group (4/4) it was shown absolute number for serum lipoproteins. [b]Based on Friedewald's equation: LDL_{CHOL} = $Total_{CHOL}$-HDL_{CHOL}-(TG/5). APOE, apolipoprotein E; LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglycerides; SD, standard deviation.

Table III. Comparison of serum lipoprotein levels and dietary fat intake between breast cancer patients and healthy controls.

Variables	Cases (n=47) Mean (25-75%)	Controls (n=165) Mean (25-75%)	P-value
Serum cholesterol (mg/dl)	193.0 (165.0-217.0)	209.0 (172.0-230.5)	0.070
Serum HDL (mg/dl)	47.0 (40.0-55.0)	50.0 (42.0-61.0)	0.166
Serum LDL (mg/dl)[a]	107.6 (79.8-140.0)	121.2 (92.7-147.0)	0.154
Serum triglycerides (mg/dl)	151.0 (105.0-200.0)	150.0 (101.5-217.5)	0.656
Dietary fat intake (g/day)	66.1 (49.4-83.0)	56.8 (45.3-67.1)	0.020
Dietary cholesterol intake (mg/day)	254.6 (197.5-325.4)	214.5 (156.8-281.2)	0.017

[a] Based on Friedewald's equation: LDL_{CHOL} = $Total_{CHOL}$-HDL_{CHOL}-(TG/5). LDL, low-density lipoprotein; HDL, high-density lipoprotein; TG, triglycerides.

such as the effect of reduced triglyceride clearance from the plasma, resulting in continuously elevated concentrations, which may result in decreased sex hormone-binding globulin levels (28). It was previously reported that e4 allele carriers may, in a dose-dependent manner, exhibit decreased ability for effective repair at the cellular level following noxious injury (3). Such an injury may also be caused by environmental factors, such as fat and cholesterol intake, alcohol consumption, stress and occupational exposures (1). This inability for repair following injury may subsequently affect serum lipids in the form of reduced triglyceride clearance from the plasma. Consequently, the serum triglyceride levels will increase, since free fatty acids and serum triglycerides routinely increase in response to trauma or during injury at the cellular level (3,4).

The allelic frequency, presented in Table IV, was generally similar to that reported by other studies. However, in our study, the e4 allele frequency in both the control (17.9%) and case groups (19.1%), was marginally higher compared to that estimated in other studies from other countries, including Brazil (Table V). A possible explanation for this difference may be the variability in ethnicities, which is characteristic of the Brazilian population. Certain studies reported that the frequency of the e4 allele may vary with different regional geography (3,27). Certain studies suggested that the e4 allele is

Table IV. Allele and genotype distribution in breast cancer patients and healthy controls.

Allele/ genotype	Cases (n=47) No. (%)	Controls (n=165) No. (%)	Total (n=212) No. (%)	P-value[a]
Alleles				0.019
e2	4 (4.2)	33 (10)	37 (8.7)	
e3	72 (76.7)	238 (72.1)	310 (73.1)	
e4	18 (19.1)	59 (17.9)	77 (18.2)	
Genotypes				0.172
e2 carriers	4 (8.5)	33 (20)	37 (17.4)	
e3 carriers	28 (59.6)	91 (55.1)	119 (56.1)	
e4 carriers	15 (31.9)	41 (24.9)	56 (26.5)	

[a]Chi-square test. The e2 carriers included the genotypes e2/e2, e2/e3 and e2/e4; the e3 carriers included e3/e3; the e4 carriers included e3/e4 and e4/e4. APOE, apolipoprotein E.

Table V. Comparison of the APOE allele frequency in women without BC (healty controls) in this study compared to that in other populations worldwide.

		APOE alleles (%)		
Authors/(Refs.)	No.	e2	e3	e4
Present study	165	10.0	72.1	17.9
Ojopi *et al* (29)	258	6.7	77.3	15.8
Chang *et al* (30)	232	9.7	75.2	15.1
Brandão *et al* (31)	118	5.1	81.4	13.5
Feng *et al* (32)	506	9.5	79.3	11.1
Schwanke *et al* (33)	70	5.0	84.0	11.0
Souza *et al* (34)	200	5.6	86.0	9.0

APOE, apolipoprotein E.

more prevalent in African-Americans compared to Hispanics or non-Hispanic whites (1) and the frequency of this allele was found to be inversely correlated with human ageing (1).

Furthermore, we identified a positive association between the presence of the e4 allele and BC (P=0.019), whereas the presence of the e2 allele was positively associated with the absence of BC. The concomitant presence of the e2 and e4 alleles was positively associated with absence of BC and e4/e4 homozygosity was positively associated with BC (P=0.021), suggesting a dominant negative effect of the e2 over the e4 allele. Evidence suggested that the e4 allele may be accompanied by less effective repair ability following DNA damage, contributing to carcinogenesis (3). The poor repair capacity associated with the e4 allele may also be due to the actual structure and function of APOE in lipid transport and cellular metabolism and differentiation (3,4). For all the lipoprotein subfractions, the e4 allele is catabolized three times faster compared to the e2 allele in heterozygous e2/e4 subjects, suggesting that these alleles may have distinct metabolic pathways (3).

Several methodological issues require consideration in interpreting our findings. Sample size was a limitation in this study, similar to other molecular epidemiological studies (5). Even having obtained a marginally higher e4 allelic frequency compared to that reported by other studies, the allelic frequencies of APOE were consistent with previous evidence (27). It is possible that the association between APOE genotype, triglycerides and BC risk may be a result of sampling variation. Another important point is that there is always a possibility that the disease process or treatment may have affected the measurements in case-control studies. It was previously hypothesized that blood lipid levels are affected by surgery as part of the metabolic and neuroendocrine response to the procedure (35). In addition, there is evidence that the blood lipid levels may be affected by chemotherapy and tamoxifen treatment (36). In this study, the BC patients exhibited lower cholesterol serum levels compared to controls (P=0.070), although the BC group reported higher fat and cholesterol dietary intake (P=0.020 and P=0.017, respectively), supporting this hypothesis.

It should be noted that the State of Rio Grande do Sul (RS, the southernmost state in Brazil) has one of the highest BC incidence rates in the country, with an estimated rate of 87 new cases per 100,000 women in 2014, a number comparable to the USA and North Europe. The State's capital, Porto Alegre, where this study was conducted, has an even higher BC incidence rate, with 146 new cases per 100,000 women projected for 2014 (37). Furthermore, in RS there is a high prevalence of cardiovascular risk factors, such as hypertension (38), obesity (39,40), smoking (39) and sedentary lifestyle (39,40), mainly among women. In this context, it is possible that the high e4 allele frequency evidenced in our study contributes not only to the high incidence rates of BC in Porto Alegre, but to the increment in the incidence of cardiovascular disease, as already reported in the literature (41).

The aim of this study was in to verify the frequency of APOE polymorphisms in a sample of women residing in an area with a high incidence of BC and cardiovascular disease. In view of the significant opposite impacts of the e4 and e2 alleles on the risk of BC, it is likely that the investigated population is more susceptible not only to BC, but also to cardiovascular diseases. As this is the first study to verify the association of APOE with BC risk in a sample of Brazilian women, the results of our study should be replicated in monoethnic and in ethnically diverse populations. The gene expression profiles in specific types of human tumors, including the expression profiles of APOE in BC, may contribute to our better understanding of the variations in the genotype of those individuals at higher risk for this disease. Such analyses may also be used to enhance the understanding of additional biological and environmental factors and may identify markers of more aggressive cells in different types of human BC.

The emphasis on achievable cancer prevention strategies with a focus on modifiable lifestyle factors is imperative, since the appropriate role of APOE genotyping for BC risk remains ambiguous, as neither the presence nor the absence of an e4 allele provides diagnostic certainty of BC susceptibility (28).

Given that there is also conflicting evidence as to whether the presence or the absence of an e4 allele confers an increased risk for BC, the diagnostic use of APOE genotyping for cancer or any other chronic disease outside of the research setting may be premature.

In conclusion, our findings suggested that APOE polymorphisms play an important role in the development of BC, particularly when associated with higher serum triglyceride levels. Overall, the APOE genotype was not found to be associated with the risk of BC, but we identified a positive association between the e4 allele and BC risk, whereas the e2 allele appeared to be protective against the disease. As the first Brazilian study to investigate the association between APOE genotype, serum lipoprotein levels and BC risk, further investigation is required to determine whether our results are replicated in monoethnic and ethnically diverse populations.

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