Apolipoprotein E polymorphism and breast carcinoma: correlation with cell proliferation indices and clinical outcome

E Zunarelli 1, JAR Nicoll 2, M Migaldi 1, and GP Trentini 1

1 Dipartimento di Scienze Morfologiche e Medico-Legali, Sezione di Anatomia Patologica, Università di Modena e Reggio Emilia, Italia; 2 Department of Neuropathology, University of Glasgow, Institute of Neurological Sciences, Southern General Hospital, Glasgow, United Kingdom

Key words: APOE polymorphism, breast tumours, clinical outcome

Summary

There is preliminary evidence that polymorphism of apolipoprotein E (apoE, protein; APOE, gene), one of the key regulatory proteins in cholesterol metabolism, influences the pathobiology of carcinoma of the colon, prostate and breast and also primary tumours of the brain. This study was designed to determine whether APOE polymorphism is related to variation in the rate of tumour cell proliferation and clinical outcome in carcinoma of the breast. One hundred and eleven infiltrating ductal carcinomas, for which follow up data were available, were included in the study. Estrogen and progesterone receptor status (ER, PR) cell proliferation index (MIB-1) and APOE genotypes were determined from paraffin-embedded tissue by standard methods. Positive correlations were found between grade and tumour size, grade and presence of metastasis, grade and MIB-1 expression, as well as between ER and PR. Survival correlated inversely with tumour size and the presence of positive lymph nodes. Both steroid receptors correlated inversely with MIB-1 expression. PR positive status also correlated inversely with high histological grade and presence of lymph node metastases. APOE allele frequencies resembled those of the general population. No significant associations were found between possession of either APOE 2 or 4 alleles and the parameters investigated. Although there is evidence to suggest that APOE 4 may predispose to the development of carcinoma of the breast our data do not support the hypothesis that APOE genotype influences the rate of tumour cell proliferation or the clinical course.

Introduction

Apolipoprotein E (apoE, protein; APOE, gene) is one of the key regulatory proteins in cholesterol and phospholipid metabolism [1–3]. In humans there are three functionally distinct isoforms of the protein (E2, E3 and E4), encoded by corresponding alleles 2, 3 and 4 [1–3].

The APOE 4 allele, carried by approximately a third of the population, is a major genetic risk factor for Alzheimer’s disease [4–6], is associated with poor outcome following traumatic brain injury and intracerebral hemorrhage [7–9] and influences the plasma lipid profile and atherosclerosis [1, 10]. Recent studies have also provided evidence of an association between APOE genotype and neoplastic disease. In a series of 35 prostatic carcinomas there was an increased frequency of homozygosity for 4 allele compared with controls and possession of the 4 allele was associated with earlier onset of the disease [11]. There is evidence that the APOE gene polymorphism influences susceptibility to adenoma and carcinoma of the proximal colon, with relative protection of patients with APOE 4 [12]. We have provided data suggesting that patients with primary brain tumors who carry APOE 4 present at an older age and have a relatively good prognosis [13]. APOE 4 allele has also been related to breast carcinoma; women with one or two copies of the 4 allele and high serum levels of tryglicerides had four times the risk of developing breast
carcinoma when compared with women with low try-
gliceride levels [14]. The mechanisms underlying the
associations described above are as yet unclear.

ApoE coordinates transport of lipids systemically
via the bloodstream and is responsible for the delivery
of cholesterol and phospholipids to cells by receptor-
mediated uptake [1–3]. There is evidence 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 recept-
ors. It has been postulated that apoE provides tumors
with the lipid substrates required for tumor growth and
that if the lipid uptake system is a rate limiting step
then tumors may grow at different rates in patients
with different APOE genotypes [15]. In this study,
therefore, we have examined a series of breast carcino-
mas, to determine if APOE polymorphism influences
the rate of tumor growth and the associated clinical
outcome.

Materials and methods

Case selection

From the archives of the Section of Anatomic Patho-
logy, University of Modena and Reggio Emilia, 111
infiltrating ductal carcinomas were selected. Cases
were restricted to this single histological subtype of
breast carcinoma in order to eliminate tumor subtype
as a variable influencing the rate of cell proliferation
and clinical outcome. All tumours were from female
patients ranging in age from 40 to 94 years (mean 66
± 12 S.D.). Lymph node dissection was performed in
all cases and the mean number of axillary lymph nodes
removed was nine (range 1–23). Follow up ranged
from 9 to 107 months (mean 85 months ± 21 S.D.);
patients were followed up according to standard clin-
ical practice. They were coded and their names were
not revealed.

The following characteristics of the tumors were
studied: stage group according to the AJCC/UICC
TNM Classification and Stage grouping [16], histolo-
gical grade according to the Elston–Ellis scheme [17],
estrogen and progesteron receptor status (ER, PR) and
cytoproliferative activity (MIB-1). These immunohis-
tochemical markers are important prognostic indica-
tors in patients with primary breast tumours, being of
value for predicting clinical behaviour in terms of re-
sponse to hormonal therapy, recurrence and survival
[18–23]. For the immunohistochemical detection of
ER and PR (Neomarker, CA, USA, prediluted mono-
clonal antibodies 6F11 and 1A6 respectively, incuba-
tion 32 minutes in toto at 40°) and MIB-1 (Bio-Optica,
Milano, Italy, prediluted monoclonal antibody Mib-1,
1 h at room temperature), the three step streptavidin-
biotin immunoperoxidase method was performed, fol-
lowing microwaving for antigen retrieval (15 min at
750 W in citrate buffer).

For analysis of ER, PR and MIB-1 immunoreact-
ivity only unequivocal nuclear staining was scored as
positive. The percentage of labelled nuclei in 500 cells
(labeling index, LI) was evaluated with the aid of
a computer assisted image analyzer (Image Pro-Plus,
version 1.1, 1994, Immagine Computer, Rho, Milano,
Italy). The cut off values for ER and PR (20%) and for
MIB-1 (15%) which we have used are those that resul-
ted from an Italian multicentric study of breast cancer.
These cut off values are used to define the appropriate
therapy according to established internal guidelines.
All specimens were evaluated without knowledge of
the clinical data.

APOE genotyping

Determination of APOE genotype was performed on
archival formalin-fixed, paraffin-embedded tumor tis-
tissue prepared for the polymerase chain reaction (PCR)
as previously described [24], blind to the other data.
Briefly, the polymorphic fragment of the APOE gene
was amplified by ‘hot start’ PCR using suitable
primers [25] followed by digestion of the PCR product
with the restriction enzyme HhaI. The products of di-
gestion were separated by polyacrylamide gel electro-
phoresis. 104/111 cases were successfully genotyped
in this way.

Statistical analysis

Statistical analysis was performed using SPSS soft-
ware (version 6.1.3, 1995, Chicago, IL, USA). The
relation between the different variables was assessed
by the Spearman’s rank correlation coefficient.

The Pearson χ² square test was used to compare
t1/T2 size, grade, N0/N1 lymph nodes, M0/M1 cases,
ER LI, PR LI, MIB-1 LI, favorable/unfavorable follow
up and presence or absence of APOE ε2 and ε4 alleles.
Associations of APOE ε2 or ε4 alleles with tumor size,
ER LI, PR LI, MIB-1 LI and survival were tested with
the one-way analysis of variance (Anova).

Survival, defined as the span between diagnosis
and death or last date of follow up, was evaluated by
the Kaplan–Meier function according to possession of