Oestrogen receptor polymorphisms are an associated risk factor for mild cognitive impairment and Alzheimer disease in women APOE ε4 carriers: a case–control study

Manuel Fernández-Martínez,1 Xabier Elcoroaristizabal Martín,2 Elisa Blanco Martín,1 Luis Galdos Alcelay,3 Iratxe Ugarriza Serrano,1 Fernando Gómez Busto,4 Maite Álvarez-Álvarez,6 Ana Molano Salazar,1 Rocío Bereincua Gandarias,1 Sandra Inglés Borda,3 Juan María Uterga Valiente,5 Begoña Indakoetxea Juanbeltz,6 María Ángeles Gómez Beldarrain,7 Josefa Moraza López,8 Myriam Barandiarán Amillano,6 Marian M de Pancorbo2

ABSTRACT

Objectives: Examine the role of single nucleotide polymorphisms (SNPs) in the oestrogen receptor (ER) genes: rs9340799, rs2234693, rs2228480 (in the ESR1 gene) and rs4986938 (in the ESR2 gene) as a risk factor for amnestic mild cognitive impairment (MCIa) and Alzheimer’s disease (AD) and its possible association with the apolipoprotein E (APOE) gene.

Design: We have investigated the independent and combined association of different alleles of the oestrogen receptor genes and APOE*ε4 allele with cognitive impairment using a case–control design.

Setting: Participants were prospectively recruited from the neurology departments of several Basque Country hospitals.

Participants: This study comprised 816 Caucasian participants who were aged 50 years and older: 204 MCIa, 350 sporadic patients with AD and 262 healthy controls.

Primary and secondary outcome measures: Clinical criteria and neuropsychological tests were used to establish the diagnostic groups (MCIa, AD and healthy controls). A dichotomous variable was used for each allele and genotype and the association with MCIa and AD was established using Logistic Regression Models.

Results: Neither alleles nor genotypes of SNPs rs9340799, rs2234693, rs2228480 and rs4986938 of oestrogen receptor genes (ESR1 and ESR2) are independently associated with the risk of MCIa or AD. However, the genetic profile created with the combination of the less represented alleles of these SNPs (expressed as XPAA*) was associated with an increased risk for MCIa (OR=3.30, 95% CI 1.28 to 8.54, p=0.014) and AD (OR=5.16, 95% CI 2.19 to 12.14, p<0.001) in women APOE*ε4 allele carriers.

Conclusions: The less represented alleles of SNPs studied are associated with MCIa and AD in APOE*E4 carriers. In particular, the genetic profile created with the less represented alleles of ESR1 and ESR2 SNPs are associated with an increased risk for MCIa and AD in women APOE*ε4 allele carriers.

INTRODUCTION

Alzheimer’s disease (AD) is the most common form of dementia, currently affecting over 9 million Americans and Europeans; its aetiology is complex and multifactorial. Several genes associated with sporadic and familial AD have been identified, but it is estimated that probably more than 50% of genetic risk remains unidentified.1

The apolipoprotein E gene (APOE) is a genetic factor closely related to the late onset AD disease, and constitutes a strong independent risk factor for sporadic AD.2 However, the APOE gene explains only a fraction of the
genetic risk associated with AD, and it is possible that other genes or metabolic factors may modify the APOE effect to initiate the pathogenesis of AD.

In the past, genetic research had focused on identifying common population polymorphism loci, such as APOE, as well as other genes such as CLU, CRI, PICALM and EXOC3L2, which have been associated with an increased risk for developing AD. These genes are implicated in chaperone action, positive regulation immune response and regulation of receptor-mediated endocytosis. Strikingly, although these genes have a significant effect on the risk of AD, risks differ by more than two orders of magnitude lower than APOE.

Oestrogens are pleiotropic hormones having an influence not only on the reproductive system but also on the central nervous system (CNS). These hormones are synthesised by ovaries and are also produced in smaller amounts by other tissues such as glia in CNS, having a wide spectrum of neuroprotective and antiapoptotic effects. 

Synaptogenic effects of estradiol-17β have been demonstrated in the adult mammalian brain (rodent and monkey models); low levels of estradiol are correlated with lower synapse density, while high estradiol levels are correlated with a higher density of synapses in the hippocampal region and dendritic spine density in CA1 pyramidal cells. Among the other positive effects of oestrogens, estradiol-17β has an effect on (1) the maintenance and increase of the neurotransmitter systems, (2) the APP processing, Aβ levels and factors that alter its clearance and aggregation and (3) mechanisms of oxidative damage. Multiple lines of evidence suggest that loss of oestrogens in the ageing brain of women and men may play a role in the cognitive declines associated with AD, but whether the female sex is also a risk factor for AD,14-18 another gene or metabolic factors may modify the genetic risk associated with AD, and it is possible that other genes or metabolic factors may modify the APOE effect to initiate the pathogenesis of AD.

Another interesting SNP is rs2228480; this polymorphism is the coding synonymous variant to codon 594 (rs2228480) within the last exon of the gene. This variant is thought to play a role in distinguishing between the receptor agonist or antagonists binding to the receptor molecule. In addition, this SNP has been associated with schizophrenia and the mechanism of this association may involve alternative gene regulation and transcript processing.

Other studies have shown an association between several polymorphisms of the ESR2 gene and the late onset AD, and they found that variations in this gene could modify disease susceptibility. The polymorphism located in the 3’UTR of the ESR2 gene, rs4986938, has been associated with the onset of Parkinson disease and the susceptibility for vascular dementia (VaD) in an Israeli cohort, but not with AD. In the study of Dresener-Pollack et al., VaD is differentiated from AD by clinical criteria, but in the absence of imaging data, the potential misclassification is high. Thus, the results should be confirmed.

Until now, no studies have been conducted in the prodromal stages of AD such as mild cognitive impairment of amnestic type (MCIa). Such studies could provide information about the beginning of the disease process, helping to ensure that suitable therapeutic measures would be implemented at an early stage. According to the above, the aim of the present study was to determine whether the ESR1 and ESR2 genes are linked to the risk of MCIa; whether there is an interaction with the APOE gene; and whether such an interaction could influence the risk of AD and MCIa. Our hypothesis is that the association of the ESR1 and ESR2 genes with cognitive impairment may exist only in the APOE*4 carriers. We have studied this association in patients with AD and in patients with MCIa, the latter condition possibly representing a prodrome for AD-type dementia.

With the purpose of examining the association of the ESR1 and ESR2 genes involved in oestrogen metabolism, as a genetic risk factor for cognitive impairment, we
conducted a study on a sample of patients with MCIa, AD and a control group. All subjects were analysed for the
ESR1 (rs9340799, rs2234693 and rs2228480) and ESR2 (rs4986938) polymorphisms and the APOE genotype.

METHODS

This study comprised 816 Caucasian participants, included in three groups: patients with MCIa (n=204),
patients with AD (n=350) and healthy controls (CTL) (n=262). Participants were prospectively recruited from
the neurology departments of several hospitals. Participants were aged 50 years and older. For patients
with AD and MCIa, evaluation also included routine blood tests: haematology, biochemistry, thyroid-
stimulating hormone, vitamin B12 levels, syphilis serology and neuroimaging test: CT scan or MRI.

The participants were evaluated using a broad battery of neuropsychological tests: Mini-Mental State
Examination (MMSE), Clinical Dementia Rating scale, CERAD protocol, Stroop test, unilateral and bilateral
motor praxis, 7 min test, Trail Making T est (TMT) Parts A & B; and neuropsychiatric inventory (NPI).

Based on the results of these evaluations, the participants were classified into the following groups: patients
with MCIa, patients with AD and healthy control subjects.

The diagnosis of patients with MCIa was based on Petersen’s criteria.40 Patients had memory complaints
corroborated by an informant, representing a decline from a previous level of functioning given their age and
educational level. The score in the CDR scale was required to be 0.5, and performance in relation to other
cognitive functions and daily living activities was required to be normal. The diagnosis of AD was based
on the DSM IV and NINCDS-ADRDA criteria for probable and possible AD. Patients with a total score of less
than three on the CDR scale (mild to moderate dementia) were included.

Healthy control subjects were scored within the normal ranges for age and educational level in psycho-
metric testing, with a CDR score of 0.

The exclusion criteria included: severe comorbidities making adequate follow-up unlikely, acute psychiatric
diseases, previous cerebrovascular diseases (transient ischaemic attacks, stroke or intracranial haemorrhage),
other neurodegenerative diseases and the absence of a reliable informant.

A specific database was designed and declared to the
Spanish Data Protection Agency. The study was approved by the Ethics Committee of Cruces Hospital (Barakaldo,
Spain). All patients signed informed consent to undergo the examination. The study was conducted in accord-
ance with the Declaration of Helsinki concerning medical research in human subjects.

Genetic analysis

On the first visit, peripheral blood samples were collected in EDTA vacuum tubes from all individuals.
Genomic DNA was extracted by proteolytic lysis from white blood cells using the standard phenol/chloroform
extraction method.

The APOE gene was amplified by PCR with 112F and 158R primers, under the PCR conditions described by
Wilton and Lim.41 Digestion of the amplified product was carried out with Hae II and Alu III, as described by
Álvarez-Alvarez et al.12

Three single nucleotide polymorphisms (SNPs) in the
ESR1 gene (rs9340799, rs2234693 and rs2228480) and one SNP in the ESR2 gene (rs4986938) were evaluated.
The first two SNPs in ESR1 (rs9340799 and rs2234693) are in intron 1 and are separated by only 46 base pairs.
The rs9340799 polymorphism marks an A→G transition 351 nucleotides upstream in intron 1 (also known as
c.454–351A>G). Those with the G allele have an absent XbaI site, which has previously been called X in the liter-
ature, with the A allele denoted by x. The rs2234693 polymorphism is characterised by a T→C transition 397
nucleotides upstream in the intron (also known as c.454–497T>C) that obliterates the PvuII restriction site.
The T allele has previously been called the p allele, while the C allele has been called the P allele, denoting
the absence of the PvuII restriction site. Subjects were described as XX, xx, PP, pp, homozygotes; and Xx or Pp
heterozygotes.

Taqman SNP Genotyping Assays were used to analyse the polymorphism rs2228480; G>A (SNP1) of ESR1 gen
and polymorphism rs4986938; G>A (SNP2) of ESR2 gen.

SNP genotypes of candidate genes (ESR1 and ESR2) and the APOE gene were analysed blinded to clinical
diagnosis.

The less frequent alleles of each SNP were evaluated, such as a combined genotype (XPAA). Therefore, with
the name of XPAA, we are referring to all haplotypes with at least one X allele (rs9340799), one P allele
(rs2234693), one A allele (rs2228480) and one A allele (rs4986938).

Statistical analyses

Genepop V.4.0 was used to test the goodness of the fit to
the Hardy-Weinberg equilibrium by means of the
Guo-Thompson exact test for all three groups studied.43

The G test was also used to check the differences
between the demographic and clinical variables, as well
as between the allele frequencies and genotype
frequencies.

Statistical analysis was also performed using the SPSS
package, V.15.0. A dichotomous variable was used for
each polymorphism: ‘yes’ or ‘no’ for ‘carrier’ or ‘non
carrier’ of the APOE*ε4 allele and for different alleles
and genotypes of the SNPs in candidate genes (ESR1
and ESR2 genes).

Several multinomial regression models were created in
order to determine the independent effects of X, P and
SNP1-A alleles of ESR1 gen and SNP2-A allele of ESR2
gen in the total sample and in the absence of the
APOE*ε4 allele. The effect of the APOE*ε4 allele in the
total sample and in the different diagnostic groups was also calculated. Another model was created to assess the combined effect of different polymorphisms of \textit{ESRI} and \textit{ESR2} genes and the APOE*\(\varepsilon\)4 allele, based on the hypothesis that the effect of oestrogens might exist only in APOE*\(\varepsilon\)4 allele carriers.

Because age and gender could be associated with the frequency of some polymorphisms, we adjusted our analysis for these covariates in the total sample. \(p\) Values of less than 0.05 were considered statistically significant.

**RESULTS**

We have investigated the independent and combined associations of \(X\), P and SNP1-A alleles of \textit{ESRI} gen and SNP2-A allele of \textit{ESR2} gen and the APOE*\(\varepsilon\)4 allele by using a case-control design.

In the present study, we analysed a sample of 204 patients with MCI, 350 patients with AD and 262 healthy control subjects without significant differences in terms of age (\(p>0.05\)). There was, however, a significant difference in the MMSE score between groups (\(p<0.05\)) (table 1). Years of education were not significantly different between groups (\(p=0.148\)).

Table 2 shows the allele and genotype frequencies of \textit{ESRI} and \textit{ESR2} polymorphisms and the APOE gene in MCI, AD and controls. In all studied groups, frequencies were in the Hardy-Weinberg equilibrium (\(p>0.05\)). There were no significant differences in the allele and genotype frequencies in MCI and AD compared to controls for \textit{ESRI} and \textit{ESR2} gene polymorphisms, while the differences proved to be significant for the APOE gene (table 3).

In order to determine whether the less represented alleles of SNPs in candidate genes (\textit{ESRI} and \textit{ESR2} genes) were an independent risk factor for MCI and AD, we selected a subgroup of individuals with MCI and AD and controls with the presence of at least one of these alleles. None of them had a significant effect (data not shown).

In the total sample, the APOE*\(\varepsilon\)4 allele is a risk factor for cognitive impairment; the ORs of developing MCI and AD were 2.44 (95% CI 1.61 to 3.69, \(p<0.001\)) and 4.23 (95% CI 2.93 to 6.12, \(p<0.001\)), respectively (table 1). Years of education were not significantly different between groups (\(p=0.148\)).

We further evaluated a possible synergistic effect between the less represented alleles of SNP in candidate genes and the APOE*\(\varepsilon\)4 allele by using a multivariate logistic regression model. To analyse this effect, we subgrouped the subjects according to the presence of X, P, SNP1-A and SNP2-A alleles and at least one APOE*\(\varepsilon\)4 allele. A slight increase in the nominal risk of MCI and AD was observed. The statistical analyses were also conducted according to gender (see supplementary tables S1 and S2).

In order to analyse the combined effect between oestrogen polymorphisms, we created a genetic profile with the less represented alleles of these SNPs, expressed as XPAA. We did not find a significant risk in the absence of one APOE*\(\varepsilon\)4 allele, but analysing the combined effect of XPAA with the APOE*\(\varepsilon\)4 allele, ORs were the following: MCI, OR=3.30 (95% CI 1.28 to 8.54, \(p=0.014\)) and AD, OR=5.16 (95% CI 2.19 to 12.14, \(p<0.001\)) compared to men.\(^{*}\) The less represented alleles of \textit{ESR1} and \textit{ESR2} genes) were independently associated with the risk of MCI or AD. The less represented alleles of SNPs in candidate genes (\textit{ESRI} and \textit{ESR2} genes) were not an independent risk factor for MCI and AD in the

**DISCUSSION**

Our study shows that neither alleles nor genotypes of SNPs rs9340799 (A>G; XbaI), rs2234693 (PvuII; C>T) and rs2228480 (A>G) (\textit{ESRI} gene) and SNP rs4986938 (A>G) (\textit{ESR2} gene) are independently associated with the risk of MCI or AD. The less represented alleles of SNPs in candidate genes (\textit{ESRI} and \textit{ESR2} genes) were not an independent risk factor for MCI and AD in the

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age*</th>
<th>Women (%)</th>
<th>MMSE‡</th>
<th>Education§</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCI</td>
<td>204</td>
<td>70.25±8.6</td>
<td>61.3</td>
<td>26.38±2.05</td>
<td>8.08±4.36</td>
</tr>
<tr>
<td>AD</td>
<td>350</td>
<td>72.17±8.3</td>
<td>71.1</td>
<td>19.68±4.60</td>
<td>8.41±7.90</td>
</tr>
<tr>
<td>CONTROLS</td>
<td>262</td>
<td>74.00±9.6</td>
<td>59.5</td>
<td>28.45±1.63</td>
<td>9.51±4.80</td>
</tr>
</tbody>
</table>

*Years, means±SD.
†Per cent of women in group.
‡MMSE score, means±SD.
§Years of education.
absence of APOE*ɛ4. Furthermore, the genetic profile created with the less represented alleles of SNPs in candidate genes were associated with an increased risk for MCIa and AD in women and APOE*ɛ4 allele carriers.

In our series, the APOE*ɛ4 allele seems to be an independent risk factor for the population with AD, and this risk is highest for women. The APOE*ɛ4 allele also constitutes a risk factor for patients with MCIa.

On evaluating the combined effect of the APOE*ɛ4 allele in the presence of alleles or genotypes of ESR1 and ESR2 SNPs the risk for AD remains significant, although this association did not confer a relevant additional risk of MCIa and AD.

When we created a genetic profile with the less represented alleles of ESR1 and ESR2 SNPs, expressed as XPAA, we did not find a significant risk in the absence of one APOE*ɛ4 allele. However, the presence of XPAA and at least one APOE*ɛ4 allele increases the risk in women with MCIa and AD.

Nowadays, the most well-known polymorphism of the ESR1 gene related to AD are SNPs rs9340799 (A>G; XbaI) and rs2234693 (PvuII; T>C). As regards the association between XbaI with AD, several studies show that the ESR1 XbaI polymorphism is an additional risk factor.32 44–46 However, other studies have not found this association.34 47–50 These results and several meta-analyses51 suggested that the ESR1 gene polymorphisms might be related to the individual susceptibility to AD, especially in women.

Several published studies have shown a great heterogeneity concerning the association between the ESR1 PvuII polymorphism and AD. In some of them, no association has been found.31 47 48 50 52 Some studies claimed a protective role for the P allele of the ESR1 PvuII polymorphism,34 45 46 whereas others found an opposite effect.32 33 44–46 However, some studies have established an association between ESR1 PP and XX genotypes with an increased risk for AD only in men (OR=3.6, 95% CI 1.2 to 10.9) and conferred a relevant additional risk of AD on subjects also carrying the APOE*ɛ4 allele and in women with AD. In this last study, the ESR1 PP and XX genotypes were also associated with lower MMSE values (p=0.0007). These data suggest that the involvement of ESR1 polymorphisms (XbaI and PvuII) in AD onset is mediated by the regulation of APOE expression. Our data support this hypothesis, in accordance with the increased risk of MCI and AD observed in patients with the APOE*ɛ4 allele.

To our knowledge, this is the first study to show evidence in support of the association of SNP rs2228480 with patients with MCI and AD and the APOE*ɛ4 allele carriers. Previously, this SNP had only been linked to the alternative regulation and transcript processing of the ESR1 gene.36 56

Until now additional information has not been provided with regard to neurodegenerative disorders.

Regarding polymorphisms of the ESR2 gene, several studies have been published with conflicting results: susceptibility for VaD but not for sporadic AD in elderly

<table>
<thead>
<tr>
<th>Table 2 Allelic and genotypic frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ESR1</strong></td>
</tr>
<tr>
<td>XbaI</td>
</tr>
<tr>
<td>Allele</td>
</tr>
<tr>
<td>X</td>
</tr>
<tr>
<td>X</td>
</tr>
<tr>
<td>Genotype</td>
</tr>
<tr>
<td>XX</td>
</tr>
<tr>
<td>Xx</td>
</tr>
<tr>
<td>xx</td>
</tr>
<tr>
<td>H-W*</td>
</tr>
<tr>
<td>p Value</td>
</tr>
<tr>
<td>PvuII</td>
</tr>
<tr>
<td>Allele</td>
</tr>
<tr>
<td>P</td>
</tr>
<tr>
<td>P</td>
</tr>
<tr>
<td>Genotype</td>
</tr>
<tr>
<td>PP</td>
</tr>
<tr>
<td>Pp</td>
</tr>
<tr>
<td>pp</td>
</tr>
<tr>
<td>H-W*</td>
</tr>
<tr>
<td>p Value</td>
</tr>
<tr>
<td><strong>ESR2</strong></td>
</tr>
<tr>
<td>SNP2</td>
</tr>
<tr>
<td>Allele</td>
</tr>
<tr>
<td>A</td>
</tr>
<tr>
<td>G</td>
</tr>
<tr>
<td>Genotype</td>
</tr>
<tr>
<td>AA</td>
</tr>
<tr>
<td>AG</td>
</tr>
<tr>
<td>GG</td>
</tr>
<tr>
<td>H-W*</td>
</tr>
<tr>
<td>p Value</td>
</tr>
<tr>
<td><strong>APOE</strong></td>
</tr>
<tr>
<td>Allele</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>Genotype</td>
</tr>
<tr>
<td>2,2</td>
</tr>
<tr>
<td>2,3</td>
</tr>
<tr>
<td>2,4</td>
</tr>
<tr>
<td>3,3</td>
</tr>
<tr>
<td>3,4</td>
</tr>
<tr>
<td>4,4</td>
</tr>
<tr>
<td>H-W*</td>
</tr>
<tr>
<td>p Value</td>
</tr>
<tr>
<td><strong>Genetic profile</strong></td>
</tr>
<tr>
<td>XPAA(+)</td>
</tr>
<tr>
<td>XPAA(–)</td>
</tr>
</tbody>
</table>

*Hardy-Weinberg probability test

Jewish women was found in the ESR2 rs4986938 polymorphism. Pirskanen et al found that some gene variants of the ESR2 gene are associated with increased risk of AD in women (rs1271573 T/T genotype and rs1256043 T/T genotype) while others are not (IVS31842, rs4986938). Lambert et al found no independent association of these polymorphisms with the risk of developing AD. One study suggests that the ESR2 allele 5 seems to be a protective factor. Meta-analyses have not been performed on the following polymorphisms of the ESR2 gene since they lack published genotype data or the published genotype data were not eligible for inclusion. Other studies have not detected a significant gene-gene interaction between ESR1, ESR2 SNPs and APOE status, but the analysis was performed in late onset AD.

In contrast with previous studies, we have analysed the genetic profile of the less represented alleles of the ESR1 and ESR2 gene polymorphisms, XPAA; when considering the XPAA isolatedly, the genetic profile was not an independent risk factor for MCIa and AD, but the combined effect with the APOE*ε4 allele confers an increased risk in women, while it does not contribute to the disease susceptibility in men. Analysis of haplotypes offers more power to detect associations than does simply focusing on a single variant, but in our case the actual results differed slightly from those expected. The combined effect observed between X, P, SNP1-A and SNP2-A alleles and at least one APOE*ε4 allele seemed to point to an increased risk in men with MCIa and women with AD. Our case-control study is relatively medium sized with a small number of samples carrying the genetic profile (<8% in patients with MCI and AD and <2% in controls) and the APOE*ε4 allele that may affect the power negatively. Nevertheless, according to our results, some variations in the ER genes in synergy with the APOE*ε4 allele may be associated with an increased risk of MCIa and AD in women.

Our results may suggest that the risk for MCIa and AD may be modulated only when the ESR1 and ESR2 genes have several polymorphisms, which might be related to their expression and biological activities. The variations in the ER genes may involve alternative gene regulation and transcript processing in the brain. The APOE gene expression can be differentially regulated depending on the activation of ER subtypes. A recent study demonstrated that activation of the ESR1 gene upregulated the APOE*ε4 mRNA and protein expression in the hippocampus. In contrast, an activation of the ESR2 gene downregulated the mRNA and protein expression of the APOE gene. Thus, it is expected to lower regulation in postmenopausal women conferring less protection against the effect of the APOE*ε4 allele.

Relatively few studies have examined the epistatic effects between the oestrogen-related pathway genes and the APOE*ε4 allele. Postmenopausal women with Down syndrome showed an increased risk of AD and an elevated sex hormone binding globulin in those carrying CYP17 and CYP19 variants and the APOE*ε4 allele. Both genes are involved in the production of neurosteroids (oestrogens and testosterone). In addition, oestrogens have been shown to affect amyloid precursor protein expression.
protein metabolism, by increasing the secretory metabolism of amyloid protein precursor (App). Oestrogens are also a potent factor that not only prevents vascular disease but also improves blood flow, including blood flow in regions of the brain affected by AD.62 Sympathetic sprouting by estradiol in a model of AD may operate via an APOE*ɛ4-dependent mechanism.63 Cholinergic neurons that are implicated in cognitive functions may be regulated by oestrogens. The distribution of ERs corresponds to that of the cholinergic system.64 The important decrease in endogenous oestrogen levels after menopause may contribute to the development of AD.65

Despite the protective effect of oestrogens on AD, this effect might be modified by ER polymorphisms, particularly in the APOE*ɛ4 allele carriers. Thus, the current state of knowledge of the role of oestrogens in preventing dementia in postmenopausal women should be reviewed.

Although the prevalence and incidence of AD are higher in women, men may also have the same effect due to SNPs in ER genes. It has been observed that while androgens have specific receptors to exert their neuroprotective action, they may also exert their actions

| Table 4 - Risk Factors for MCI and AD from Logistic Regression Models |
|--------------------------|--------------------------|--------------------------|--------------------------|
|                         | MCI OR CI 95% p Value    | AD OR CI 95% p Value      |
| **Global effects**       |                          |                          |
| X (+)†                   | 1.39 (0.93 to 2.06) 0.104| 1.18 (0.85 to 1.67) 0.324 |
| P (+)‡                   | 1.25 (0.82 to 1.90) 0.293| 1.26 (0.88 to 1.23) 0.205 |
| SNP1-A§                  | 1.14 (0.76 to 1.71) 0.506| 1.13 (0.78 to 1.62) 0.510 |
| SNP2-A¶                  | 1.05 (0.71 to 1.54) 0.304| 1.08 (0.77 to 1.51) 0.649 |
| E4 (+)**                 | 2.44 (1.61 to 3.69) <0.001| 4.23 (2.93 to 6.12) <0.001|
| Women                    | 1.07 (0.73 to 1.56) 0.705| 1.67 (1.19 to 2.35) 0.003 |
| E4 (+)*Women††           | 2.27 (1.32 to 3.87) 0.003| 4.85 (3.04 to 7.73) <0.001|
| E4 (+)*Men‡‡             | 2.74 (1.43 to 5.23) 0.002| 3.19 (1.73 to 5.88) <0.001|
| **Independent effects**  |                          |                          |
| X (+) E4(–)§§            | 1.04 (0.65 to 1.66) 0.863| 1.18 (0.76 to 1.81) 0.452 |
| P (+) E4(–)§§            | 0.86 (0.52 to 1.40) 0.545| 1.19 (0.754 to 1.90) 0.444 |
| SNP1-A(+) E4(–)§§        | 1.19 (0.74 to 1.92) 0.469| 1.13 (0.73 to 1.76) 0.568 |
| SNP2-A(+) E4(–)§§        | 1.03 (0.65 to 1.66) 0.879| 1.07 (0.70 to 1.64) 0.758 |
| **ESR1**                 |                          |                          |
| E4(+)X§§                 | 3.17 (1.80 to 5.59) <0.001| 5.07 (3.00 to 8.55) <0.001|
| E4(+)P¶¶                 | 2.74 (1.55 to 4.85) 0.001| 5.35 (3.11 to 9.17) <0.001|
| E4(+)SNP1-A¶¶           | 2.53 (1.31 to 4.90) <0.001| 4.44 (2.48 to 7.93) <0.001|
| **Combined effects**     |                          |                          |
| E4(+)SNP2-A¶¶           | 2.77 (1.55 to 4.93) 0.001| 4.87 (2.91 to 8.17) <0.001|

**Genetic profile (XPAAX)**

| **Independent effects**  |                          |                          |
| XPAA*E4(–)***            | 1.31 (0.48 to 3.54) 0.590| 1.19 (0.49 to 2.91) 0.696 |
| XPAA(–)*E4(+)†††         | 2.53 (1.61 to 3.93) <0.001| 4.32 (2.91 to 6.40) <0.001|
| **Combined effects**     |                          |                          |
| XPAA*E4(+)§§§            | 3.30 (1.28 to 8.54) 0.014| 5.16 (2.19 to 12.14) <0.001|
| XPAA*E4(+)Women§§§       | 3.84 (1.09 to 13.57) 0.036| 8.04 (2.60 to 24.80) <0.001|
| XPAA*E4(+)Men§§§         | 3.20 (0.73 to 14.11) 0.124| 3.57 (0.88 to 14.47) 0.075|

†Effect of sample with at least one X of RFLP XbaI.
‡Effect of sample with at least one P of RFLP PvuII.
§Effect of sample with at least one A allele of rs2228480.
¶Effect of sample with at least one A allele of rs4986938.
**Effect of sample with at least one E4 allele of the APOE gene.
††Women selected by at least one E4 allele of the APOE gene.
‡‡Men selected by at least one E4 allele of the APOE gene.
§§Sample selected by at least one allele that is indicated and the absence of the E4 allele of the APOE gene.
¶¶Sample selected by at least one E4 allele of the APOE gene and one of the alleles that is indicated. The reference category was sample control.
***Sample selected by absence of the E4 allele of the APOE gene and the presence of XPAA.
†††Sample selected by absence of XPAA and the presence by at least one E4 allele of the APOE gene. Sample selected by at least one E4 allele of the APOE gene and the presence XPAA.
‡‡‡Sample selected by at least one E4 allele of the APOE gene and the presence of XPAA.
§§§Women or men selected by at least one E4 allele of the APOE gene and the presence of XPAA. * In all models, the reference category was sample control considering the age and sex (as appropriate).
indirectly via CYP17 by aromatisation of testosterone to estradiol or directly through the binding of the metabolite dihydrotestosterone to ESR2. Until now, it is unclear whether SNPs in ER genes would increase the risk of AD or MCI in men. Our partial data show a tendency to increase the risk of MCI in men. Future studies should elucidate whether there is a relationship between ER genes and MCI in men.

The strengths of our study are its multicenter nature including patients with AD, healthy controls and patients with MCI. To our knowledge, our study is the first to investigate an association between polymorphisms of ER (rs9340799, rs2234693, rs2228480 and rs4986938) and cognitive function not only in patients with AD, but also in patients with MCI. Moreover, the patient sample is not small, allowing gender stratification.

Some limitations in our study must be addressed. The study population comes from the hospital setting. A community-based study could provide more information. The serum levels of estradiol have not been measured, and we do not know whether the patients received ERT in the last years. We also include a sample of patients with MCI; this stage is probably a heterogeneous clinical entity, but the broad battery of neuropsychological tests used in our sample might ensure the highest homogeneity.

CONCLUSIONS

In our study, the APOE*4 allele is an independent risk factor for patients with MCI and AD. The combined effect of the APOE*4 allele and the less represented alleles of ESR1 and ESR2 SNPs remains the risk for MCI and AD; this association confers a relevant additional risk of AD and MCI in women and men, respectively. Nevertheless, the genetic profile with the less represented alleles of the ESR1 and ESR2 gene polymorphisms, expressed as XPAA, did not increase the risk of cognitive impairment in the absence of one APOE*4 allele, but the presence of XPAA and at least one APOE*4 allele only increases the risk in women with MCI and AD.

Author affiliations
1Department of Neurology, Hospital Universitario Cruces, BioCruces Health Research Institute, Barakaldo, Bizkaia, Spain
2BIOMICS Research Group, Department of Z and Cellular Biology A, Centro de Investigación y Estudios Avanzados Lucio Lascaray (CIEA), University of Basque Country UPV/EHU, Vitoria-Gasteiz, Álava, Spain
3Department of Neurology, Hospital Universitario Txagorritxu, Vitoria-Gasteiz, Álava, Spain
4San Prudencio Comprehensive Care Center for Elderly, Vitoria-Gasteiz City Council Basque Country, Vitoria-Gasteiz, Álava, Spain
5Department of Neurology, Hospital Universitario Basurto, Bilbao, Vizcaya, Spain
6Department of Neurology, Hospital Universitario Donostia, Donostia, Guipuzcoa, Spain
7Department of Neurology, Hospital de Galdakao, Galdakao, Vizcaya, Spain
8Department of Neurology, Hospital Santiago Apóstol, Vitoria-Gasteiz, Vizcaya, Spain

Contributors MFM conceived the study and participated in its design and coordination, as well as drafting the manuscript. XEM participated in the study design and coordination and helped to draft the manuscript. EBM, IUS and MAA were involved in the drafting of the manuscript. LGA and FGB were involved in the study design and coordination and helped to draft the manuscript. AMS, RGB, SIB, NO, MBA and MCZ performed the battery of neuropsychological tests. JMV, BJU, MAGB and JML participated in the design of the study and coordination of the manuscript. MMF participated in the study design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Funding This work was sponsored by grants from the Federación de Asociaciones de Familiares de enfermos de Alzheimer de Euskadi, Fondo de Investigación Sanitaria del Instituto Carlos III (Madrid), Pfizer Foundation and Ayudas a la Investigación de la Obra Social de la Caja Vital Kuba.

Competing interests None.

Patient consent Obtained.

Ethics approval Approved by the Ethics Committee of Cruces Hospital (Barakaldo, Spain).

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

Open Access This is an Open Access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 3.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/3.0/

REFERENCES


Oestrogen receptor polymorphisms are an associated risk factor for mild cognitive impairment and Alzheimer disease in women APOE ?4 carriers: a case –control study


BMJ Open 2013 3:

doi: 10.1136/bmjopen-2013-003200

Updated information and services can be found at:

http://bmjopen.bmj.com/content/3/9/e003200

These include:

Supplementary Material
Supplementary material can be found at:
http://bmjopen.bmj.com/content/suppl/2013/09/18/bmjopen-2013-003200.DC1

References
This article cites 61 articles, 6 of which you can access for free at:
http://bmjopen.bmj.com/content/3/9/e003200#ref-list-1

Open Access
This is an Open Access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 3.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/3.0/

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections

- Genetics and genomics (113)
- Geriatric medicine (313)
- Neurology (446)

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/