## PEER REVIEW HISTORY



## ARTICLE DETAILS

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| **TITLE (PROVISIONAL)** | Estrogen receptor polymorphisms are an associated risk factor for mild cognitive impairment and Alzheimer disease in women APOE ε4 carriers |
| **AUTHORS** | Blanco, Elisa; Fernández-Martínez, Manuel; Elcoroaristizabal, Xabier; Galdós, Luis; Ugarriza-Serrano, Iratxe; Gómez-Busto, Fernando; Álvarez-Álvarez, Maite; Molano, Ana; Bereincua, Rocío; Inglés, Sandra; Uterga, Juan; Indakoetxea, Begoña; Gómez-Beldarraín, María; Moraza, Josefa; Barandiarán, Myriam; Martinez-De Pancorbo, Marian |

## VERSION 1 - REVIEW

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| **REVIEWER** | Nicole Schupf, Ph.D.<br>Professor of Clinical Epidemiology<br>Taub Institute for Research on Alzheimer's disease and the Aging Brain |
| **REVIEW RETURNED** | 14-Jun-2013 |

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| **GENERAL COMMENTS** | In this study, the authors examine the relation of SNPS in the genes for estrogen receptors, ESR1 and ESR2, to risk of MCIa and AD in men and women. The literature on the role of estrogen receptor variants in affecting risk for AD is inconsistent and only a few papers have examined the combined effects of polymorphisms in both genes and both sexes. The strengths of the paper include examination of MCIa as well as AD in both men and women and the analyses of combined genotypes. However there are several concerns with the design of the study.<br><br>1. The findings of a relationship between the ERS1 and ESR2 polymorphisms and AD are not novel, and have been described in several studies already, including the increased strength of an association in APOE E4 carriers (see : Ji et al., 2000.; Brandi ML et al., ,. 1999; Corbo RM et al., 2006;. Mattila KM et al., Neurosci Lett 2000)<br><br>2. The primary concern is the very limited number of SNPs on each gene that have been genotyped. These are fairly large genes and the study does not provide good coverage, but instead focuses on replication and extension to combined genotypes, with MCIa and AD, of SNPS that have already been shown to be involved. Other SNPS may well influence risk for AD, but are not examined here and some have been shown to be active in other studies. For example, Pirskanen et al (2005) found that rs1256065 , rs1271573, rs1256043) were associated with increased risk for cognitive impairment or AD in women but not men . Yaffe et al, (2009) found that rs1255998 was associated with increased risk in men but not women. Zhao et al, in addition to a significant association |

with rs4986938, found another block of SNPS,rs17766755, rs4365213, rs12435857 in introns 6 and 7 at some distance from rs4986938, that were also associated in a prospective cohort study with increased risk for incident AD in postmenopausal women with Down Syndrome. A more systematic and complete coverage of these genes could have advanced understanding of the role of these genes in AD. Given that allele frequencies and linkage disequilibrium patterns may differ in the study population from those in groups studied by other investigators, better coverage of the genes would have provided a better test of the association of estrogen related variants to cognitive decline and risk of AD.

3. This appears to be a cross sectional study, although it is noted that the participants were "prospectively recruited" from the neurology departments of several hospitals. Was this true of the controls as well?. It may be that the effect of these variants is on age at onset rather than overall risk—ie the effect is to decrease/increase age at onset. Thus examination of the association of these SNPs with incident MCI or incident AD , using a cox proportional hazard approach would be more informative. The cross sectional nature of the study makes it difficult to support the contention that there is no independent association of these SNPs with risk for MCI or AD because effects on age at onset cannot be examined.

4. The analysis models are not well described. It appears that age and gender were included in some, but not all, models? It would be important to include education as well. An analysis of the independent effects of the ESR1 and ESR2 SNPS, adjusting for the presence/absence of an e4 allele would have been helpful

5. The tables show many combined groups, but the n's for each group are not presented. So it is not clear to what extent failure of these SNPs to reach statistical significance is due to small sample size and low power.

6. Use of sex-stratified analyses would have been more informative. In addition, Table 4 shows a significant OR for AD for the E$9+)*men interaction terms. Also analyses stratified by e4 might be more informative

7. As the authors note, use of a hospital based study population may not represent the general population and may introduce biases. Hospital/Clinical based groups are generally better educated and have less comorbidity than community based study groups. Thus their primary risk factor may be the APOE e4 allele, as it is well known that the frequency of the e4 allele is higher in clinic- based AD cases than in community-based AD cases, while other risk factors for AD ( eg diabetes) are found at lower frequencies . This possibility is supported by the high OR's found for the e4 allele.

Minor points

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| | 1. Several abbreviations, eg EA, are not defined.<br>2. Educational level for the three groups should be presented and education should be included in the analysis |

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| **REVIEWER** | Finch, Caleb<br>University of Southern California, Gerentology |
| **REVIEW RETURNED** | 03-Jul-2013 |

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| **THE STUDY** | Novelty: May be the first study of estrogen receptor Snp associations with prodromal stages of AD.<br>1. Needs careful editing for grammar, punctuation, and syntax errors.<br>2. Some statements are imprecise: “ERs are located through around the brain”, by which the authors may mean : Ers are expressed in neurons and glia throughout the brain.<br>3. rs2228480…has been associated with neurodegenerative disorders (31). This reference refers to schizophrenia, and has not been verified or extended to other brain dysfunctions.<br>4. The abbreviation EA was not defined, presumably for early AD. Please do not introduce unnecessary new terminology. The Intro should define other terms: VaD, MMSE.<br>5. Female risk: Ref 3 (1997) has been superceded by many reports that did not find gender differences. Nonetheless, authors could cite mouse ADtg studies which generally show great amyloid and neurodegeneration in females.<br>6. State % of AD variance explained by apoE snps and alleles: give range from major studies.<br>7. For estrogen effects on synapses, make clear that data are from rodent and monkey models.<br>8. For HT effect controversies, update refs 20-21(2009).<br>9. Concept of xp/XP was not in the introduction.<br>10. Cite other studies which found other AD candidate SNP associations with ApoE4 but not other APoE alleles.<br>11. Please comment on the gender specificity of these interactions |

## VERSION 1 – AUTHOR RESPONSE

We were pleased to have an opportunity to revise our paper entitled, “Estrogen receptor polymorphisms are an associated risk factor for mild cognitive impairment and Alzheimer disease in women APOE ε4 carriers”. In revising the paper, we have carefully considered comments and suggestions of the reviewers. As instructed, we have attempted to succinctly explain changes made in reaction to all comments. After providing a brief overview of ways in which the paper was revised, we reply to each comment in a point-by-point fashion. The reviewer’s comments were very helpful and we are appreciative of such constructive feedback on our original submission. After addressing the issues raised, we feel the quality of the paper is much improved and hope you agree.

First, authors are going to answer the concerns of Nicole Schuph (R1) and after that Caleb Finch´s (R2).

R1.1: We appreciative of your comments. As you say one of the strengths of the paper include examination of MCIa as well as AD, and although the findings of a relationship between the ERS1 and ESR2 polymorphisms and AD are not novel, we also think that the originality of this paper is the inclusion on MCI patients. So in our knowledge this is the first paper to analyze the role of estrogenic polymorphism in MCI, AD and healthy controls.

R1.2: Our focus is on the most important polymorphisms related to the risk to develop AD, taking into account the number of patients we have chosen the most representative polymorphism. Technical reason not allowed us to include more patients and more SNPs.

We consider very interesting your appreciations about other SNPs in ERS1 and ERS2 so we included some references about this point commenting the gender differences showed in them. We agree with you about the interest of examining a Down syndrome population, but this is not the main objective of our paper however we have revised the bibliography and we have included two references about it.

R1.3: All the participants were prospectively recruited from different neurological departments, and this is also true for controls. The controls also underwent all clinical and neuropsychological procedures.

R1.4: In all models sex and gender have been included, because significant differences were found, obviously in those not specified. Following your comment, we have included the sentence “In all models reference category was sample control considering the age and sex (as appropriate).” This is not the case for education level: years of scholarship were similar in all groups (p= 0,148). We have included the following phrase“Years of education were not significantly different between groups (p=0,148)” and we have incluided the education information in table 1.
We feel now that the result can be clearly understood. Thank you for noting this.

R1.5: Thank you for this suggestion. We have created the Supplementary Table 2, (see it in the main document). This table allows knowing the percentage of the samples that are represented with the test done. Furthermore, we have included the new sentence “Supplementary table 2 shows the size of samples that carry the genetic characteristic considered in the input of combined models in all groups. Overall, significant differences between the control frequencies and patient’s frequencies provided enough power to address this question.” in the result part.

R1.6: Following your comment, we have calculated all models taking into account the sex-stratified. These results allowed us identified that women had a slight trend to increase the OR in AD and men in MCI. Outcomes are interesting, the table that collects this information is presented in Suplementary table 1 (Risk Factors for combined effects in MCI and AD from Logistic Regression Models). Furthermore, taking into account that we have performed a regression analyses corrected by sex and age the inclusion of another results could be redundant. Thus, we feel that could be best add this phrase in results part “The statistical analyzes were also conducted according to the gender (Supplementary table 1). A significant increased OR was found between the X, P, SNP1-A and SNP2-A alleles tested and MCI men, but it has not been clear observed in women. The opposite effect was observed in the AD group, women showed a greater OR than men.”

Respect to the stratified by E4 allele, we have included in “independent effect and combined effect” in table 4. So we feel that this issue referred for the reviewer has been included in the present table. If these are issues that you feel remain to be addressed, we would welcome an opportunity to do so.

Overall, we believe that now it is easier for the reader understand the relationship between the risk of AD and MCI and estrogens receptor according to the gender and the effect of E4 allele.

R1.7: As pointed out by the reviewer hospital based study population have clear limitations, and may not represent general population. Nevertheless, the majority of these studies are based on hospital groups of patients and are not representative of the real world. It also very known that the frequencies of E4 allele is higher in clinical records, probably due to the fact that patients with memory problems have a trend to ask for medical care and to be carriers of this allele.

R1.m1: Done.
R1.m2: See answer R1.4.

Thank you for your comments that have improved the paper. We hope you will view our revision attempt positively.

R2.1: Done.
R2.2: Done
R2.3: We have change the sentence: “In addition, this SNP has been associated with schizophrenia and the mechanism of this association may involve alternative gene regulation and transcript processing”
R2.4: Done.
R2.5: We have introduced a new reference about the controversial point whether female sex is also a risk factor (1) and we have cited mouse ADtg studies which show great amyloid and neurodegeneration in females (2,3)

R2.6 A.Thank you for this suggestion. We have created the Supplementary Table 2 (see the main document), this table allows knowing the percentage of the samples that are represented with the test done. Furthermore, we have included the new sentence “Supplementary table 2 shows the size of samples that carry the genetic characteristic considered in the input of combined models in all groups. Overall, significant differences between the control frequencies and patient’s frequencies provided enough power to address this question for a minimum detectable OR between 2.0 and 5.” in the result part.

R2.7. Done.

R2.8: Done (4,5)

R2.9 We have explained this concept in the introduction as you suggested “There are several polymorphic loci in intron 1 of ESR1 gen, highlighting the PvuII and XbaI locus (6). The polymorphisms of PvuII were coded as P or p and the polymorphisms of XbaI as X or x, in which the capital letter signifies the absence of the restriction site and the lower case letter signifies its presence.”

R2.10+ R2.11: We have included the following paragraph according to both previous comments “Relatively few studies have examined the epistatic effects between estrogen-related pathway genes and APOE*ε4 allele. Postmenopausal women with down syndrome showed an increased risk of AD and elevated sex hormone binding globulin in those carrying CYP17 and CYP19 variants and APOE*ε4 allele(7). Both genes are involved in the production of neurosteroids (estrogens and testosterone). […..] “Although the prevalence and incidence of AD are higher in women, men also may have same effect dur to SNPs in ER genes. It has been observed that while androgens have specific receptors to exert its neuroprotective action, also they may exert their actions indirectly via CYP17 by aromatization of testosterone to estradiol(8) or directly through ESR2 binding capacity of the metabolite dihydrotestosterone(9). To date, it is unclear whether SNPs in ER genes would

increase the risk of AD or MCIa men. Our partial data trend to increase the risk of MCIa in men, although the data seems to indicate otherwise. Future studies should elucidate whether there is a relationship between ER genes and MCIa men."

Thank you for your comments that have improved the paper. We hope you will view our revision attempt positively.

Bibliografy included in responses to reviewers

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3. Wang J, Tanila H, Puolivali J, Kadish I, van Groen T. Gender differences in the amount and deposition of amyloidbeta in APPswe and PS1 double transgenic mice. Neurobiol Dis 2003;14(3):318-27.
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7. Chace C, Pang D, Weng C, Temkin A, Lax S, Silverman W, et al. Variants in CYP17 and CYP19 cytochrome P450 genes are associated with onset of Alzheimer's disease in women with down syndrome. Journal of Alzheimer's disease : JAD. 2012;28(3):601-12. Epub 2011/11/08.
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9. Lund TD, Hinds LR, Handa RJ. The androgen 5alpha-dihydrotestosterone and its metabolite 5alpha-androstan-3beta, 17beta-diol inhibit the hypothalamo-pituitary-adrenal response to stress by acting through estrogen receptor beta-expressing neurons in the hypothalamus. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2006;26(5):1448-56. Epub 2006/02/03.

**VERSION 2 – REVIEW**

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| **REVIEWER** | Caleb E Finch<br>ARCO Professor in the Neurobiology of Aging<br>Davis School of Gerontology<br>University of Southern California<br>Los Angeles CA, USA<br><br>no competing interests |
| **REVIEW RETURNED** | 05-Aug-2013 |

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| **GENERAL COMMENTS** | Ready to publish with minor corrections<br>downs to downs<br>decimals: identify by periodnnot comma: 11,1 change to 11.1 |