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Estrogen receptor polymorphisms and incident dementia: the prospective 3C study

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ABSTRACT

Background: Genetic variation in the estrogen receptor (ESR) may be associated with the incidence of Alzheimer's disease, but this association could be modified by genetic and environmental factors.

Methods: The association between five ESR alpha (ESR1) and beta (ESR2) polymorphisms with 7-year dementia incidence was examined among 6959 older men and women from the Three City Study using multivariate-adjusted Cox regression models with delayed-entry. Gender, the apolipoprotein E (ApoE) ε4 allele and hormone treatment were considered as potential effect modifiers of this association.

Results: Among women, the CC genotype of ESR1 *rs2234693* was associated with a small increased risk of AD specifically (adjusted HR: 1.54, 95% CI: 1.03-2.28, p=0.03). However, women with this genotype had a substantially increased risk of AD associated with the Apoe-ε4 allele (adjusted HR: 3.24, 95% CI: 1.81-5.79 for women *rs2234693* CC, compared to HR: 1.87, 95% CI: 1.37-2.56 for all women). There was also evidence of a nominally significant interaction between the ESR1 and ESR2 on the risk of all dementias (p=0.04). Hormone treatment did not modify these associations and there were no significant associations in men.

Conclusions: While there was only weak support for a gender-specific association between the common ESR1 *rs2234693* polymorphism and AD, this polymorphism may act as an effect modifier, modifying the association between an ESR2 polymorphism and dementia, as well as the risk of AD associated with the Apoe-ε4 allele.

Key words

Alzheimer's disease; Dementia; Estrogen receptor polymorphisms; ESR1; ESR2; Gender-Specific; ApoE; Epidemiology; Cohort Study.

1. Background

Dementia is a multi-factorial disease which has been associated with a large number of interacting environmental, biological and genetic risk factors. Recent genome-wide association studies (GWAS) have identified a few candidate genes for Alzheimer's disease (AD) [1, 2], however together these genes only explain a small amount of the underlying genetic component of the disease [3]. This may be accounted for in part by the interplay between genetic and environmental factors, in complex gene-gene and gene-environment interactions that cannot be identified through GWAS.

Epidemiological studies have reported that women have a higher incidence of dementia, and in particular AD compared to men [4], which suggests the potential involvement of steroid hormones such as estrogen. In support of this, estrogen is synthesised in the brain by the aromatization of androgens with estrogen receptors (ESR) being present in limbic brain regions known to be implicated in dementia [5], and there is considerable experimental evidence indicating that estrogen has neuroprotective and neurotrophic effects [6]. Furthermore, a number of cohort and case-controlled studies have found that the risk of dementia and especially AD was reduced in postmenopausal women using estrogen-containing hormone treatment, although this was not supported by the findings of a large randomised controlled trial (see for review [7]). It is plausible that genetic variants which modify estrogen signalling, such as polymorphisms in the estrogen receptors alpha (ESR1) and beta (ESR2), could be candidate risk genes for dementia.

Indeed, a number of case-control studies have examined differences in the frequency of ESR1 polymorphisms between patients with late-onset AD and controls, but the exact association remains unclear. Some of these studies have reported that AD patients had a significantly higher frequency of the minor C and G alleles of *rs2234693* and *rs9340799* respectively [8-11], yet other studies have found no significant associations [12-14] or even reverse associations [15, 16]. Meta-analyses conducted a few years ago found a small but significant association between the minor alleles of these polymorphisms and an increased risk of AD (OR~1.2) [17, 18], however this is not supported by the most recent meta-analysis on the Alzheimer's Research Forum (http://www.alzgene.org). Gender-specific effects have not been examined in these analyses, nor have gene-gene interactions been considered. There are limitations to case-control studies however, which are inherently more prone to selection bias and a

greater risk of population stratification which is of particular concern for genetic association studies. The only prospective study to be undertaken [19], the Rotterdam study of 2483 men and 3573 women aged at least 55 years, failed to find a significant association between the two common ESR1 variants and the 6-year risk of all-cause dementia or AD [19]. This finding is yet to be replicated in another cohort. Furthermore, very few studies overall have investigated associations between ESR2 polymorphisms and dementia, despite evidence from animal studies suggesting it plays a key neuroprotective role [20].

The present study examines the association between ESR1 polymorphisms and the risk of all-cause dementia or AD in the elderly general population. As only the second prospective study to be undertaken, we aim to help clarify previous findings, while also investigating prospectively for the first time, potential associations with ESR2 polymorphisms. Based on previous findings from relatively small case-control studies we also investigated *a priori* interactions between ESR1 and ESR2 receptors on the risk of dementia [21], and the possibility that ESR polymorphisms could further increase the risk of AD associated with the apolipoprotein E (ApoE) ε4 allele [9, 11].

2. Methods

2.1 Study participants

The Three City Study (3C) is a multi-centre longitudinal study of community-dwelling elderly aged 65 years and over from three French cities [22]. Recruitment of the study cohort from the electoral rolls took place between 1999 and 2001. The study protocol was approved by the Ethical Committee of the University- Hospital of Kremlin-Bicêtre (France). Written informed consent was obtained from all participants in the study. At baseline and the 2, 4 and 7-year follow-ups, participants were administered standardised questionnaires by trained staff and underwent clinical examinations. Of the 9080 dementia-free participants recruited to the 3C Study, 644 refused to provide blood samples for genotyping analysis and 670 had no follow-up data. A further 474 had incomplete genotyping data and 333 had missing data for at least one of the covariates considered in this analysis. This data is thus based on 6959 men and women. Compared to the analysed sample, participants not included in this analysis were more likely to

be older, have a lower education level, physical incapacities, depressive symptoms and comorbidity (p-values<0.005) at baseline and they were also more likely to be diagnosed with dementia during the follow-up period (p<0.001). There was no significant difference between excluded and included participants in terms of the frequency of the Apoe-ε4 allele or of the ESR1 and ESR2 genotypes, with the exception of the *rs4986938*, where excluded participants were more likely to carry the variant A allele (p=0.02).

2.2 Dementia diagnosis

Dementia diagnosis was based on a three-step procedure [22], the first of which involved a thorough neuropsychological examination by trained psychologists, including the assessment of different aspects of cognitive function. The severity of cognitive disorders, activities of daily living, and, where possible, magnetic resonance images or computed tomography scans were also collected. All participants suspected of having dementia were then examined by a neurologist. The final step of the diagnosis involved a review of all potential cases of dementia by a national panel of independent neurologists who were experts in the field of dementia. Cases were reviewed using all of the existing information and a consensus on the diagnosis of dementia was obtained according to DSM-IV revised criteria and etiology. Alzheimer's disease was classified according to the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS/ADRDA) criteria [23]. This current study focused on the 7-year incidence of dementia, and participants diagnosed with possible or probable dementia during the baseline examination were thus excluded.

2.3 Genotyping

Fasting venous blood samples were taken from the participants at baseline. DNA was extracted from white blood cells (Puregene kit, Qiagen, France) and stored at -80°C. Genotyping of the Apoe-ε4 allele was performed at a genotyping facility in Lille, France (http://www.genopole-lille.fr/spip/). Based on the combination of restriction fragment length polymorphism bands, participants carrying at least one copy of the Apoe-ε4 allele were identified. Genotyping of ESR polymorphisms was performed by Kbiosciences

(Hoddesdon Herts, UK) using their competitive allele-specific PCR Single-Nucleotide Polymorphism (SNP) genotyping system (KASPar). The amplified PCR products were analysed by fluorescence scanning in a BMG labtech Pherastar scanner and the results were interpreted with KlusterCaller 1.1 software. The error rate for the KASPar assay system is less than 0.3%.

The two most commonly studied *ESR1* polymorphisms were examined (http://www.alzgene.org) [17, 24], *rs2234693* and *rs9340799* (otherwise known as *Pvu*II and *Xba*I), which are located at position 397 and 351 of intron 1 respectively. There is some evidence that these polymorphisms may be functionally significant [14, 25, 26], or at least are in linkage disequilibrium with a functional polymorphism elsewhere in the gene [19]. We examined these polymorphisms separately, rather than the haplotype, given that some dementia studies have reported an association with only one of these polymorphisms [11, 16]. Three *ESR2* polymorphisms with unknown functional consequences but showing potential causal associations with other hormone-related health outcomes [27, 28] were investigated: *rs1271572* in the promoter region, *rs1256049* (position 1082 of exon 5) and *rs4986938* (position 1730 in the 3'-untranslated region of exon 8). *Rs4986938* is also the only ESR2 polymorphism which has been examined on more than one occasion in prior case-control studies of prevalent dementia [21, 29].

2.4 Covariates

Information was gathered at baseline on the participant's age, education level, consumption of alcohol and smoking status. Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Women recorded current hormone treatment use which was validated by presentation of the prescription or the medication itself. The Center for Epidemiologic Studies Depression Scale (CES-D) [30] was used for the assessment of depressive symptoms (CES-D $\geq$16). Participants were classified as having moderate to severe activity limitations if they were unable to complete at least one activity from both the Rosow and Breslau mobility and the Instrumental Activities of Daily Living scales [31, 32]. Information on the health of the participants was obtained through detailed medical questionnaires, a complete inventory of drug use in the preceding month and from fasting blood samples. Participants were

classified as having comorbidity if they suffered from one or more of the following chronic illnesses: vascular diseases (angina pectoris, myocardial infarction, stroke, cardiovascular surgery, bradycardia or palpitations), asthma, diabetes (fasting glucose≥7.0mmol/l or reported treatment), hypercholesterolemia (total cholesterol ≥6.2mmol/l), hypertension (resting blood pressure ≥160/95mm Hg or treated), thyroid problems or cancer diagnosis within the last two years.

2.5 Statistical Analysis

Chi-squared tests were used to compare the distribution of ESR genotypes with those predicted under the Hardy-Weinberg equilibrium and pair-wise linkage disequilibrium was estimated. Baseline characteristics of participants associated with dementia incidence were identified using chi-squared tests. Cox proportional hazard models with delayed entry were then used to assess the gender-specific association between ESR polymorphisms and the 7-year incidence of dementia/AD in men and women. To avoid the non-proportionality in dementia risk with age, the time scale used in the models was age [33]. Time at entry into the study was defined as age at recruitment, and time at exit was defined as age of diagnosis (for cases) or age at censoring (for at-risk subjects). Crude and multivariate models were calculated. Multivariate analysis controlled for covariates significantly associated with dementia and which could potentially confound the relationship between dementia and ESR polymorphisms. The *a priori* interactions that were considered in this analysis were based on previous reports in the literature. This included an interaction between ESR1 and ESR2 polymorphisms [21] and the potential for ESR polymorphisms to modify the association between the Apoe-ε4 allele and the risk of AD [9, 11]. We also examined potential interactions with hormone treatment, as it has been shown to modify the association between ESR and other health outcomes [34-36], but no prior study has examined whether it can modify the risk of dementia. SAS v9.2 was used for the analyses (SAS Institute, Inc., North Carolina).

3. Results

The study sample comprised 4256 women and 2703 men free of dementia at baseline, with an age range of 65 to 100 years. Around 15% of the women were current users of hormone treatment. Over 39,589

person-years, with a median of 6.6-years per person (interquartile range: 3.9-7.2 years), there were 471 cases of all-cause dementia (6.8%) and 321 (4.6%) were classified as AD. Compared to non-demented participants, those participants who were diagnosed with incident dementia were more likely to be older, to have a lower education level, and to be a carrier of the Apoe-ε4 allele (Table 1). In addition, participants with incident dementia were more likely to have moderate or severe activity limitations, depressive symptoms and comorbidity at baseline and were more likely to use anticholinergics compared to those without dementia. The incidence of all-cause dementia was identical between males and females (6.8%).

The ESR genotype frequencies in the overall population are shown in Tables 2 and 3. Given that the A allele of *rs1256049* was rare, the AA (n=11) and GA (n=560) genotypes were grouped for all subsequent analysis. The ESR frequencies were not significantly different from those predicted by the Hardy-Weinberg equilibrium, except in the case of *rs1271572* (χ^2_1=4.3, p=0.04). The two ESR1 SNPs were in strong linkage disequilibrium (|D'|=0.98), as were the three ESR2 SNPs (|D'|>0.90 for all pairwise comparisons).

In multivariate adjusted Cox proportional hazards models there was no significant association between ESR2 *rs1256049, rs1275172* or *rs4986938* and the risk of all-cause dementia or AD among the male or female participants (Table 2). Gender-stratified analysis also indicated no significant association between the ESR1 polymorphisms and the risk of all-cause dementia or AD in men (Table 3). On the other hand, in women a significant association was found between *rs2234693* and the risk of AD specifically, with the CC genotyping being associated with a 1.5-times increased risk (95% CI: 1.03-2.28). After correction for multiple testing however (p<0.007), this association would not remain significant. There was a weak non-significant trend for the closely-linked GG genotype of *rs9340799* to be associated with a similar increased risk of AD. We also considered the potential for *rs2234693* to modify the risk of AD associated with the Apoe-ε4, by including an interaction term in the models (p=0.068), and subsequent stratified analyses indicated that the CC genotype of *rs2234693* further increased the risk of AD associated with the Apoe-ε4 (Table 4). Across all women, Apoe-ε4 was associated with a 1.87 times increased risk of AD in women (95% CI: 1.37-2.56, p<0.0001). When stratified by *rs2234693* however, in multivariate adjusted analysis, we found that women with the CC

genotype had a 3.2-fold increased risk of AD associated with the Apoe-ε4 ($p<0.0001$). For women with the TT genotype, the Apoe-ε4 was associated with a 1.9-fold increased risk which just failed to reach significance ($p=0.06$) and for women with the CT genotype there was no significantly increased risk of AD associated with the Apoe-ε4 allele ($p=0.12$). Further adjustment for BMI or the current use of hormone treatment or did not modify the significance of any of the associations described above and there were no significant interactions between ESR1 and either BMI or hormone treatment.

Based on the findings of a sole previous study [21], we then examined whether ESR1 *rs2234693* could interact with any of the ESR2 polymorphisms to modify the risk of dementia in women, and found a nominally significant interaction with *rs1256049* on the risk of dementia ($p=0.04$). Indeed, while *rs1256049* was not associated with dementia risk across all women (HR: 1.34, $p=0.14$), it was significantly associated with a 2.5-fold increased risk of dementia in women carrying the TT genotype of *rs2234693* (HR: 2.66, 95% CI: 1.38-5.11, $p=0.003$). By stark contrast, no significant association was found in women who were carrying either of the other genotypes (CT or CC) (Table 5). Reversing this analysis and stratifying by *rs1256049*, the CT and CC genotypes of *rs2234693* appeared to be associated with an increased risk of dementia in women with *rs1256049* GG genotype only (data not shown). Thus the combination of either ESR2 *rs1256049* GG and ESR1 *rs2234693* C allele or *rs1256049* A allele and *rs2234693* TT was associated with an increased dementia risk. There were no significant interactions between *rs2234693* and either *rs1271572* or *rs4986938*.

4. Discussion

In this large prospective population-based study we have found only weak evidence for an association between common ESR1 polymorphisms and the incidence of AD in women, although *rs2234693* may act as an effect modifier, modifying the association between an ESR2 polymorphism and dementia, as well as the risk of AD associated with the Apoe-ε4 allele.

A number of case-control studies have compared the frequency of ESR1 genotypes between patients with late-onset AD and controls and have reported significant associations (see for review [24]), but there have been some inconsistencies concerning which polymorphisms or even alleles of common

polymorphisms are associated with an increased risk. Other case-control studies have found no significant associations [12-14], although the small sample sizes in several cases meant a lack of statistical power. There are also some limitations to case-control studies, which have inherently higher selection bias and a greater risk of population stratification. We have attempted to clarify and extend these findings by following 6959 elderly men and women over seven years and examining the association between ESR1 and ESR2 polymorphisms and the incidence of all-cause dementia or AD. Based on our sample size, the incidence of all-cause dementia (6.8%), and the frequency of the ESR genotypes, our study could detect an risk ratio of at least 1.5 (between 1.39 and 1.49) comparing homozygous for the minor and major alleles, assuming a significance level of 0.05 and 80% power.

We found that women with the CC genotype of *rs2234693* had a 1.5-times increased risk of AD at nominal significant levels. This association would not remain significant after correction for multiple testing. There was a similar trend for the GG genotype of *rs9340799* to increase the risk of AD in women. The direction of these associations is supported by a majority of significant case-control studies [8-11, 37], although several prior studies have reported larger effect sizes in smaller samples. These differences may be partly explained by the populations studied, as the majority of significant findings have been found in Japanese populations [10, 11, 37]. Although these ESR1genotype frequencies are relatively similar between Caucasian and Asian populations, ethnic differences in linkage disequilibrium between these polymorphisms and other functional polymorphisms is highly likely [38]. This may account for the variability in the strength of the associations with dementia. Failure to adjust for potential confounding factors in these case-control studies might also have influenced their results.

The weak association reported in our study was specific to AD as we found no evidence of an association with all-cause dementia. This is supported by the findings of the only other prospective study [19] and suggests that estrogen may influence the pathogenic processes of AD specifically. Furthermore, the association between ESR1 polymorphisms and AD was gender-specific, with a significant association found in women only. In men, the hazard ratios for the female “risk genotypes” was actually below 1.0, indicating a non-significant tendency for these alleles to be associated with a reduced rather than increased risk. A prior study of older people reported a similarly reversed association in men and women when they investigated estrogen levels and cognitive function [39]. This suggests that estrogen can have

differential effects in older women compared to men, and may relate to the fact that postmenopausal women have experienced a marked decline in endogenous estrogen levels from their pre- to postmenopausal state. Such a change in estrogen levels is not observed in men. Indeed, age-related loss of estrogen is a female-specific risk factor for AD [40]. Finally, it is possible that these gender differences can be partly explained by sex dimorphism in brain morphology, neurochemistry and neuronal wiring (see for review [41]).

We found no significant associations between three ESR2 polymorphisms and the risk of all-cause dementia or AD. Of the few prior case-control studies which have examined ESR2 polymorphisms [13, 21, 29, 42, 43], *rs4986938* is the only one common to multiple studies. In keeping with our findings, they report no independent association between AD prevalence and *rs4986938*. The only other study to examine non-AD dementia however, found the A allele of *rs4986938* was more frequent in women with vascular dementia (n=60), compared to controls (n=68) [42]. Furthermore, in one of the case-control studies, a significant interaction between *rs4986938* and ESR1 *rs2234693* or *rs9340799* was found to modify the risk of AD in univariate analysis (p=0.04 and 0.03 respectively) [21]. Unfortunately due to the small sample size (186 cases and 405 controls), they could not assess whether this interaction was gender-specific. In our study we also found some evidence of an ESR2-ESR1 interaction at a similar level of significance (p=0.04). The A allele of ESR2 *rs1256049* was associated with a multivariate-adjusted increased risk of all-cause dementia, but only for women who were homozygous for the major allele of ESR1 *rs2234693* (HR: 2.66, 95%CI: 1.38-5.11, p=0.003). Such an interaction between the two estrogen receptor subtypes could have a biological sense, even though the functional significance of these particular polymorphisms remains unknown. *In vitro* studies have shown that ESR transcriptional activation and the response to estrogens is influenced by the relative levels of ESR1 and ESR2 [44] and the ability of these receptor subtypes to form heterodimers and act cooperatively [45]. This potentially interesting finding thus warrants further investigation and confirmation. The only other prior study to examine potential ESR1-ESR2 interactions, reported no significant association, but this was based on their adjusted significance level of <0.0003, given that they investigated 195 possible interactions (39 ESR1 and 5 ESR2 SNPs) [43].

The ApoE gene is the most consistent genetic risk factor for late-onset AD, with the ε4 allele being associated with an increased risk. Our study presents evidence of a cumulative effect between the Apoe-ε4 and the CC genotype of ESR1 *rs2234693* on the risk of AD in women, although the interaction fell short of statistical significance (p=0.068). Across all women, the Apoe-ε4 allele was associated with a highly significant 1.9 times increased risk (p<0.0001), however in women with the CC genotype, the risk of AD with the Apoe-ε4 allele increased to 3.2-fold (p<0.0001). This observation is supported by findings from two case-control studies [8, 9], which grouped participants based on their ESR1 and ApoE-ε4 genotypes and compared the prevalence of AD between groups. A third case-control study also showed that the CC genotype of *rs2234693* was more frequently in AD patients with the ApoE-ε4 than in those without [11]. Previous case-control studies which have directly tested for an ESR1 x ApoE-ε4 interaction however, have failed to find a significant association (p>0.10) [15, 16, 21, 43]. Notably however, most of these null findings have accompanied studies which have also failed to find an independent association between *rs2234693* and AD [14, 19, 21, 43]. Findings from animal studies do suggest that estrogen and ApoE can interact in the brain. Using an AD mouse model, the ability of estradiol to enhance synaptic sprouting was dependent on the presence of ApoE [46] and estrogen depletion can cause an increase in Aβ accumulation and this could be reversed by estrogen treatment [47]. Estrogen has also been shown to increase ApoE mRNA levels and thus upregulates expression of the ApoE gene [48], and this occurred in large part through ESR1 [48]. Previous research from epidemiological studies also suggests an interaction between estrogen and ApoE, as estrogen treatment can attenuate the increased risk of AD associated with the ApoE-ε4 allele [49, 50].

Limitations to our study include the bias introduced from excluding participants with missing data who were in poorer health at baseline and were significantly more likely to be diagnosed with dementia during follow-up, thus reducing the overall power of the study. There is also the possibility of population stratification which we could not control for because French law prohibits the collection of data related to ethnicity. However, genotype frequencies for participants with incident dementia and non-demented participants were similar to those observed previously in white Europeans [51]. The results presented here were not adjusted for multiple comparisons and they would fail to remain significant after Bonferroni correction. However, there was a strong *a priori* biological rationale for investigating these

specific associations and interactions. Following from this, the fact that we did examine only a small number of SNPs to limit the risk of type 1 errors, means that we may have missed potential associations between SNPs in other areas of the ESR1 or ESR2 genes and the risk of dementia. Thus we cannot draw global conclusions regarding the involvement of these genes in the disease. Our study is strengthened by its design and population-based sample. This is only the second and the largest prospective study to examine associations with ESR1 polymorphisms and incident dementia, which was monitored over a longer follow-up period. Furthermore, it is the first prospective study to investigate potential associations with ESR2 polymorphisms. Dementia was diagnosed based on DSM-IV criteria and validated by a panel of independent neurologists. The inclusion of a large number of men and women has enabled the assessment of gender-specific associations, which were not considered by many previous studies. The size of the dataset and the vast information relating to each participant also enabled adjustment for an extensive range of socio-demographic, health and lifestyle variables, and our study was adequately powered to detect moderate effect sizes. This contrasts with previous studies which have predominantly presented only age-matched analyses in small populations and have failed to consider potentially important confounding factors like hormone treatment. Furthermore, only a few prior case-control studies have investigated *a priori* gene-gene interactions, as we have done here, and ours is the first study to examine whether hormone treatment can act as an effect modifier in the association between ESR polymorphisms and dementia. Such gene-gene and gene-environment interactions cannot be tested in GWAS or meta-analyses.

Over the last 15 years there has been a considerable amount of research focused on the detection of genetic variants associated with dementia. The ApoE-ε4 is the only consistent and established genetic risk factor for the most common form of dementia, late-onset AD. It is likely that a number of other genetic factors are involved but they may be missed in individual studies or GWAS with stringent statistical thresholds and consequently low power to detect associations [3]. Large scale meta-analyses which combine results from multiple studies, such as the Alzheimer Research Forum [17] (http://www.alzgene.org) are therefore important for the identification of other genetic risk factors, in particular those with strong *a priori* biologically hypotheses indicating their involvement in the disease. In terms of ESR, most previous association studies have been undertaken using small specific populations

within a case-control design, which have obvious limitations and thus require conformation. Our multi-centric prospective study of almost 7000 participants has enabled us to examine for the first time the gender-specific association between ESR1 and ESR2 polymorphisms with the 7-year risk of all-cause dementia and AD, while taking into account potentially important factors such ApoE-ε4, comorbidity and hormone treatment. Our results suggest just a weak association with the *rs2334693* polymorphism, specific for AD and in women only. We also present some evidence that this polymorphism may act as an effect modifier, modifying the association between an ESR2 polymorphism and dementia, as well as the risk of AD associated with the Apoe-ε4 allele, but these findings require replication in another large population-based prospective study.

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Conflicts

Dr. Ritchie serves on scientific advisory boards for the Biomedical Research Centre, King's College London, and London and MRC Strategic Steering Committee (Longitudinal Health and Aging Research Unit). All other authors report no conflicts of interest.

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Abbreviations

ESR: estrogen receptor; ESR1: estrogen receptor alpha; ESR2: estrogen receptor beta; AD: Alzheimer's disease; GWAS: genome-wide association study; ApoE: apolipoprotein E; BMI: Body Mass Index; CES-D: Center for Epidemiologic Studies Depression Scale.

References

[1] Hollingworth P, Harold D, Sims R, Gerrish A, Lambert JC, Carrasquillo MM, et al. Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. Nat Genet, 2011;43:429-35.

[2] Seshadri S, Fitzpatrick AL, Ikram MA, DeStefano AL, Gudnason V, Boada M, et al. Genome-wide analysis of genetic loci associated with Alzheimer disease. Jama, 2010;303:1832-40.

[3] Altshuler D, Daly MJ, Lander ES. Genetic mapping in human disease. Science, 2008;322:881-8.

[4] Fratiglioni L, Launer LJ, Andersen K, Breteler MM, Copeland JR, Dartigues JF, et al. Incidence of dementia and major subtypes in Europe: A collaborative study of population-based cohorts. Neurologic Diseases in the Elderly Research Group. Neurology, 2000;54:S10-5.

[5] Osterlund MK, Hurd YL. Estrogen receptors in the human forebrain and the relation to neuropsychiatric disorders. Prog Neurobiol, 2001;64:251-67.

[6] Spencer JL, Waters EM, Romeo RD, Wood GE, Milner TA, McEwen BS. Uncovering the mechanisms of estrogen effects on hippocampal function. Front Neuroendocrinol, 2008;29:219-37.

[7] Ryan J, Scali J, Carriere I, Ritchie K, Ancelin ML. Hormonal treatment, mild cognitive impairment and Alzheimer's disease. Int Psychogeriatr, 2008;20:47-56.

[8] Brandi ML, Becherini L, Gennari L, Racchi M, Bianchetti A, Nacmias B, et al. Association of the estrogen receptor alpha gene polymorphisms with sporadic Alzheimer's disease. Biochem Biophys Res Commun, 1999;265:335-8.

[9] Corbo RM, Gambina G, Ruggeri M, Scacchi R. Association of Estrogen Receptor alpha (ESR1) PvuII and XbaI Polymorphisms with Sporadic Alzheimer's Disease and Their Effect on Apolipoprotein E Concentrations. Dement Geriatr Cogn Disord, 2006;22:67-72.

[10] Isoe-Wada K, Maeda M, Yong J, Adachi Y, Harada H, Urakami K, et al. Positive association between an estrogen receptor gene polymorphism and Parkinson's disease with dementia. Eur J Neurol, 1999;6:431-5.

[11] Ji Y, Urakami K, Wada-Isoe K, Adachi Y, Nakashima K. Estrogen receptor gene polymorphisms in patients with Alzheimer's disease, vascular dementia and alcohol-associated dementia. Dement Geriatr Cogn Disord, 2000;11:119-22.

[12] Combarros O, Riancho JA, Arozamena J, Mateo I, Llorca J, Infante J, et al. Interaction between estrogen receptor-alpha and butyrylcholinesterase genes modulates Alzheimer's disease risk. J Neurol, 2007;254:1290-2.

[13] Giedraitis V, Kilander L, Degerman-Gunnarsson M, Sundelof J, Axelsson T, Syvanen AC, et al. Genetic analysis of Alzheimer's disease in the Uppsala Longitudinal Study of Adult Men. Dement Geriatr Cogn Disord, 2009;27:59-68.

[14] Maruyama H, Toji H, Harrington CR, Sasaki K, Izumi Y, Ohnuma T, et al. Lack of an association of estrogen receptor alpha gene polymorphisms and transcriptional activity with Alzheimer disease. Arch Neurol, 2000;57:236-40.

[15] Boada M, Antunez C, Lopez-Arrieta J, Caruz A, Moreno-Rey C, Ramirez-Lorca R, et al. Estrogen receptor alpha gene variants are associated with Alzheimer's disease. Neurobiol Aging, 2011.

[16] Monastero R, Cefalu AB, Camarda C, Noto D, Camarda LK, Caldarella R, et al. Association of estrogen receptor alpha gene with Alzheimer's disease: a case-control study. J Alzheimers Dis, 2006;9:273-8.

[17] Bertram L, McQueen MB, Mullin K, Blacker D, Tanzi RE. Systematic meta-analyses of Alzheimer disease genetic association studies: the AlzGene database. Nat Genet, 2007;39:17-23.

[18] Luckhaus C, Sand PG. Estrogen Receptor 1 gene (ESR1) variants in Alzheimer's disease. Results of a meta-analysis. Aging Clin Exp Res, 2007;19:165-8.

[19] den Heijer T, Schuit SC, Pols HA, van Meurs JB, Hofman A, Koudstaal PJ, et al. Variations in estrogen receptor alpha gene and risk of dementia, and brain volumes on MRI. Mol Psychiatry, 2004;9:1129-35.

[20] Wang L, Andersson S, Warner M, Gustafsson JA. Morphological abnormalities in the brains of estrogen receptor beta knockout mice. Proc Natl Acad Sci U S A, 2001;98:2792-6.

[21] Lambert JC, Harris JM, Mann D, Lemmon H, Coates J, Cumming A, et al. Are the estrogen receptors involved in Alzheimer's disease? Neurosci Lett, 2001;306:193-7.

[22] The 3C Study Group. Vascular factors and risk of dementia: Design of the three city study and baseline characteristics of the study population. Neuroepidemiology, 2003;22:316-325.

[23] McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology, 1984;34:939-44.

[24] Sundermann EE, Maki PM, Bishop JR. A review of estrogen receptor alpha gene (ESR1) polymorphisms, mood, and cognition. Menopause, 2010;17:874-86.

[25] Herrington DM, Howard TD, Brosnihan KB, McDonnell DP, Li X, Hawkins GA, et al. Common estrogen receptor polymorphism augments effects of hormone replacement therapy on E-selectin but not C-reactive protein. Circulation, 2002;105:1879-82.

[26] Alonso P, Gratacos M, Segalas C, Escaramis G, Real E, Bayes M, et al. Variants in estrogen receptor alpha gene are associated with phenotypical expression of obsessive-compulsive disorder. Psychoneuroendocrinology, 2011;36:473-83.

[27] Rexrode KM, Ridker PM, Hegener HH, Buring JE, Manson JE, Zee RY. Polymorphisms and haplotypes of the estrogen receptor-beta gene (ESR2) and cardiovascular disease in men and women. Clin Chem, 2007;53:1749-56.

[28] Ryan J, Scali J, Carriere I, Scarabin PY, Ritchie K, Ancelin ML. Estrogen receptor gene variants are associated with anxiety disorders in older women. Psychoneuroendocrinology, 2011;36:1582-6.

[29] Pirskanen M, Hiltunen M, Mannermaa A, Helisalmi S, Lehtovirta M, Hanninen T, et al. Estrogen receptor beta gene variants are associated with increased risk of Alzheimer's disease in women. Eur J Hum Genet, 2005;13:1000-6.

[30] Radloff L. The CES-D scale: a self-report depression scale for research in the general population. Appl Psychol Measurement, 1977;1:385-401.

[31] Lawton MP, Brody EM. Assessment of older people: self-maintaining and instrumental activities of daily living. Gerontologist, 1969;9:179-86.

[32] Rosow I, Breslau N. A Guttman health scale for the aged. J Gerontol, 1966;21:556-9.

[33] Commenges D, Letenneur L, Joly P, Alioum A, Dartigues JF. Modelling age-specific risk: application to dementia. Stat Med, 1998;17:1973-88.

[34] Abbas S, Brauch H, Chang-Claude J, Dunnebier T, Flesch-Janys D, Hamann U, et al. Polymorphisms in genes of the steroid receptor superfamily modify postmenopausal breast cancer risk associated with menopausal hormone therapy. Int J Cancer, 2010;126:2935-46.

[35] Herrington DM, Howard TD, Hawkins GA, Reboussin DM, Xu J, Zheng SL, et al. Estrogen-receptor polymorphisms and effects of estrogen replacement on high-density lipoprotein cholesterol in women with coronary disease. N Engl J Med, 2002;346:967-74.

[36] Ryan J, Scali J, Carriere I, Peres K, Rouaud O, Scarabin PY, et al. Oestrogen receptor polymorphisms and late-life depression. Br J Psychiatry, 2011;199:126-31.

[37] Urakami K, Wakutani Y, Wada-Isoe K, Yamagata K, Adachi Y, Nakashima K. [Analysis of causative genes and genetic risk factor in Alzheimer's disease]. Nihon Ronen Igakkai Zasshi, 2001;38:769-71.

[38] Gennari L, Merlotti D, De Paola V, Calabro A, Becherini L, Martini G, et al. Estrogen receptor gene polymorphisms and the genetics of osteoporosis: a HuGE review. Am J Epidemiol, 2005;161:307-20.

[39] Hogervorst E, De Jager C, Budge M, Smith AD. Serum levels of estradiol and testosterone and performance in different cognitive domains in healthy elderly men and women. Psychoneuroendocrinology, 2004;29:405-21.

[40] Barron AM, Pike CJ. Sex hormones, aging, and Alzheimer's disease. Front Biosci (Elite Ed), 2012;4:976-97.

[41] Gillies GE, McArthur S. Estrogen actions in the brain and the basis for differential action in men and women: a case for sex-specific medicines. Pharmacol Rev, 2010;62:155-98.

[42] Dresner-Pollak R, Kinnar T, Friedlander Y, Sharon N, Rosenmann H, Pollak A. Estrogen receptor beta gene variant is associated with vascular dementia in elderly women. Genet Test Mol Biomarkers, 2009;13:339-42.

[43] Goumidi L, Dahlman-Wright K, Tapia-Paez I, Matsson H, Pasquier F, Amouyel P, et al. Study of Estrogen Receptor-alpha and Receptor-beta Gene Polymorphisms on Alzheimer's Disease. J Alzheimers Dis, 2011.

[44] Pettersson K, Delaunay F, Gustafsson JA. Estrogen receptor beta acts as a dominant regulator of estrogen signaling. Oncogene, 2000;19:4970-8.

[45] Pace P, Taylor J, Suntharalingam S, Coombes RC, Ali S. Human estrogen receptor beta binds DNA in a manner similar to and dimerizes with estrogen receptor alpha. J Biol Chem, 1997;272:25832-8.

[46] Stone DJ, Rozovsky I, Morgan TE, Anderson CP, Finch CE. Increased synaptic sprouting in response to estrogen via an apolipoprotein E-dependent mechanism: implications for Alzheimer's disease. J Neurosci, 1998;18:3180-5.

[47] Zheng H, Xu H, Uljon SN, Gross R, Hardy K, Gaynor J, et al. Modulation of A(beta) peptides by estrogen in mouse models. J Neurochem, 2002;80:191-6.

[48] Srivastava RA, Srivastava N, Averna M, Lin RC, Korach KS, Lubahn DB, et al. Estrogen up-regulates apolipoprotein E (ApoE) gene expression by increasing ApoE mRNA in the translating pool via the estrogen receptor alpha-mediated pathway. J Biol Chem, 1997;272:33360-6.

[49] Rippon GA, Tang MX, Lee JH, Lantigua R, Medrano M, Mayeux R. Familial Alzheimer disease in Latinos: interaction between APOE, stroke, and estrogen replacement. Neurology, 2006;66:35-40.

[50] Ryan J, Carriere I, Scali J, Dartigues JF, Tzourio C, Poncet M, et al. Characteristics of hormone therapy, cognitive function, and dementia: the prospective 3C Study. Neurology, 2009;73:1729-37.

[51] Ioannidis JP, Stavrou I, Trikalinos TA, Zois C, Brandi ML, Gennari L, et al. Association of polymorphisms of the estrogen receptor alpha gene with bone mineral density and fracture risk in women: a meta-analysis. J Bone Miner Res, 2002;17:2048-60.