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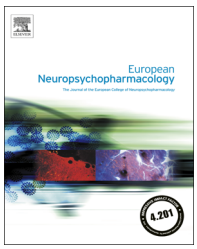

SHORT COMMUNICATION

Prospective analysis of the association between estrogen receptor gene variants and the risk of cognitive decline in elderly women

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Abstract
A plethora of data suggests a role for estrogen in cognitive function and genetic variants in the estrogen receptors *ESR1* and *ESR2* have been implicated in a range of hormone-sensitive diseases. It remains unknown however, whether *ESR* polymorphisms are associated with the risk of decline in specific domains of cognitive function. Data came from 3799 non-demented, community-dwelling elderly women recruited in France to the 3C Study. A short cognitive test battery was administered at baseline and 2, 4 and 7 years follow-up to assess global function, verbal fluency, visual memory, psychomotor speed and executive function. Detailed socio-demographic, behavioral, physical and mental health information was also gathered and genotyping of five common *ESR1* and *ESR2* polymorphisms was also performed. In multivariable-adjusted Cox analysis, *ESR1 rs2234693* and *rs9340799* were not significantly associated with the risk of decline on any of the cognitive tasks. However, significant associations with *ESR2* polymorphisms were identified. The A allele of *rs1256049* was associated with an increased risk of substantial decline in visual memory (HR:1.64, 95% CI: 1.23–2.18, $p=0.0007$), psychomotor speed (HR:1.43, 95% CI: 1.12–1.83, $p=0.004$), and on the incidence of Mild Cognitive

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Impairment (HR:1.31, 95% CI: 1.05–1.64, $p=0.02$). There was also a weaker association between the A allele of *rs4986938* and a decreased risk of decline in psychomotor speed. Our large multicentre prospective study provides preliminary evidence that *ESR2* genetic variants may be associated with specific cognitive domains and suggests that further examination of the role of this gene in cognitive function is warranted.

1. Introduction

Estrogen has been extensively studied for its actions in the brain and a plethora of data indicates its involvement in cognition (Brann et al., 2007). Through predominantly cellular and rodent studies, estrogen has been implicated in neurogenesis and synaptogenesis and has been shown to modulate various neurotransmitter systems (e.g. dopaminergic, cholinergic, serotonergic systems) and influence synaptic plasticity (Craig and Murphy, 2007; Hojo et al., 2008). Epidemiological and clinical studies also provide some evidence that endogenous estrogen and exogenous treatment could be associated with cognitive function, although this remains controversial and may be lifestage-dependent (Ancelin and Ritchie, 2005; Henderson, 2010). Estrogen receptors *ESR1* and *ESR2* are located throughout the brain (Osterlund and Hurd, 2001) and could play a key role in estrogen's effect on cognitive function. Activation of either *ESR* subtype can help prevent neurodegeneration in hippocampal neurons (Zhao et al., 2004) and can mediate the estrogen's effect on synaptic plasticity and learning (Wilson et al., 2013).

Variants in the *ESRs* have been associated with other hormone-sensitive diseases (Domingues-Montanari et al., 2008; Ioannidis et al., 2002; Ryan et al., 2011) and a few studies have investigated the association between *ESR1* polymorphisms and cognitive performance (den Heijer et al., 2004; Olsen et al., 2006; Yaffe et al., 2002, 2009). While some significant findings have been reported, these studies focused almost exclusively on cognitive impairment or global function. Prospective studies examining decline in specific domains of cognition are lacking.

We investigated the association between five previously examined *ESR1* and *ESR2* polymorphisms with the 7-year risk of cognitive decline using tasks that assessed global function, visual memory, verbal fluency, psychomotor speed and executive function. Based on the literature, we hypothesized that there would be only a weak association between these *ESR1* variants and global cognitive decline, but a stronger association with decline on specific cognitive tasks.

2. Experimental procedures

2.1. Participants

The cohort for the Three City Study was recruited between 1999 and 2001, by randomly selecting eligible people (aged over 65 years and non-institutionalised) from the electoral rolls in three French cities (The 3C Study Group, 2003). The study protocol was approved by the Ethical Committee of the University—Hospital of Kremlin-Bicêtre and all participants provided written informed consent.

2.2. Assessment of cognitive function

Cognitive tests designed to assess different areas of cognitive function, as detailed previously (Ryan et al., 2009), were administered by trained staff at baseline and each follow-up interview (2, 4 and 7 years). The Mini-Mental State Examination (MMSE) measured global cognitive performance and Benton's Visual Retention Test (BVRT) assessed visual memory. Isaacs Set Test provided a measure of verbal fluency or semantic access (number of items produced within 30 s). The Trail Making tests A (TMTA) and B (TMTB) assessed psychomotor speed and executive function respectively. In these timed visual motor tasks, participants need to connect consecutively numbered circles (TMTA) or alternate number and letter circles (TMTB). Mild Cognitive Impairment (MCI) was defined as an education and aged matched score in the lowest quintile on at least one cognitive test, and with a cognitive complaint (detailed previously (Artero et al., 2006)). A panel of expert neurologists reviewed all incident cases independently from study investigators. We considered the date of MCI onset to be the date of the follow-up interview at which it was diagnosed.

2.3. Genotyping

DNA extracted from blood cells was stored at −80 °C. *ESR* genotyping was performed by Kbiosciences (Hoddesdon Herts, UK) using their competitive allele-specific PCR Single-Nucleotide Polymorphism genotyping system (KASPar), which has an error rate of $<0.3\%$. Amplified PCR products were analyzed by fluorescence scanning and results interpreted with KlusterCaller 1.1 software. The most commonly studied *ESR1* polymorphisms, *rs2234693* and *rs9340799* located at positions 397 and 351 of intron 1, were examined, and they may be functionally significant (Maruyama et al., 2000). Three *ESR2* polymorphisms showing associations with other hormone-related health outcomes (Ioannidis et al., 2002; Ryan et al., 2011) were investigated: *rs1256049* (position 1082, exon 5), *rs4986938* (position 1730, exon 8, 3′-UTR) and *rs1271572* (promoter region). *APOE-ε4* genotyping allele was performed 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.

2.4. Covariates

Information was obtained on the participant's age, education level, consumption of alcohol, smoking status use of hormone treatment. Body mass index was calculated (weight, kg/height squared, m^2). The Centre for Epidemiology Studies Depression Scale (CES-D) was used to assess depressive symptoms (Radloff, 1977). Participants with activity limitations were unable to complete at least one activity from both the Rosow and Breslau mobility and the Instrumental Activities of Daily Living scales (as detailed previously (Ryan et al., 2011)). Through detailed medical questionnaires, a complete drug inventory and fasting blood samples, comorbidity was defined as having at least one of the following chronic illnesses: vascular diseases (angina pectoris, myocardial infarction, stroke, cardiovascular surgery, bradycardia or palpitations), asthma, diabetes (fasting glucose ≥7.0 mmol/l or reported

treatment), hypercholesterolemia (total cholesterol ≥6.2 mmol/l), hypertension (resting blood pressure ≥160/95 mm Hg or treated) or thyroid problems.

2.5. Analysis

Cognitive decline was evaluated over the 7-year period by calculating the change in test scores between each of the follow-ups (2, 4 or 7 years) and the baseline score. Given the non-normal distribution of scores on the cognitive tests and as described previously (Ancelin et al., 2012), cognitive decline was defined as being in the highest quintile of the difference in score (substantial decline) which persisted over follow-up. Specifically, the cut-offs used to define "persistent cognitive decline" corresponded to a decline ≤3 points on MMSE and BVRT and ≤10 points on Isaacs Set Test, with no more than a 1 point increase in scores from baseline (improvement) at subsequent follow-ups. For the timed trail making tasks, cut-offs used were ≥11 s on the TMTA (with ≥9 s compared to baseline at subsequent follow-ups) and 57 s on the TMTB (with ≥40 s compared to baseline at subsequent follow-ups). Only persistent decline was examined to minimize misclassification which would occur when a women performed poorly on a cognitive tasks at one follow-up, but scored highly at all subsequent visits. However, secondary analysis was used to confirm that any significant associations remained, even if participants with fluctuating scores were included in the analyses. The time of decline was the first visit follow-up examination when the women fell below the cut-off. Cox proportional hazards models with delayed entry were used to assess whether ESR polymorphisms were associated with the risk of cognitive decline. Age was used as the time scale in the models to avoid the non-proportionality in risk of decline with age in the elderly (Thiebaut and Benichou, 2004). This model enabled us to take into account the full information available until time of censoring, either by death or loss to follow-up, or the time of observed cognitive decline, thus minimizing selection bias due to cohort attrition. This approach was preferred over mixed model analysis which may be sensitive to minor and fluctuating changes in cognitive scores over time, including learning effects from repeated cognitive tests. Multivariate models adjusted for potentially confounding variables, as well as baseline cognitive performance. Poor baseline performance was defined as scoring in the lowest quintile for each cognitive test or the highest quintile for TMTA and TMTB. The potential effect modification by hormone treatment and the *APOE-ε4* allele was considered by including appropriate *ESR*-interaction terms in the models. SAS (v9.2) was used for all analyses (SAS Institute, Inc., North Carolina) with a 5% significance level. The Bonferroni-adjusted *p*-value was 0.0017.

3. Results

Of 5526 dementia-free women recruited to the 3C Study, 440 had incomplete baseline cognitive testing and 643 refused to provide blood samples or had incomplete genotyping. Over the 7 years, 371 had no follow-up cognitive testing or were lost to follow-up. A further 156 participants were missing covariate data and 117 had fluctuating cognitive scores. Excluded women were older, less educated, more frequently depressed, and with activity limitations and comorbidity ($p<0.005$). There were no significant differences between excluded and included participants however, in *ESR1* and *ESR2* genotypes, or in the risk of decline on Isaacs, BVRT, TMTA and TMTB.

Table 1 presents the baseline characteristics of the 3799 women included in this study. Older women at baseline were significantly more likely to decline on each of the cognitive tasks or to have incident MCI over follow-up (Supplementary Table S1). A lower level of education and more frequent health problems were also common among women with cognitive decline. The distribution of *ESR* genotypes in this population did not deviate significantly from Hardy-Weinberg equilibrium: *rs2234693* $\chi^2=0.48$, $p=0.49$; *rs9340799* $\chi^2=1.34$, $p=0.25$; *rs1256049* $\chi^2=1.01$, $p=0.31$; *rs4986938* $\chi^2=1.71$, $p=0.19$; except in the case of *rs1271572* $\chi^2=3.86$, $p=0.05$ (Table 2).

The Cox proportional hazards models for the association between *ESR* polymorphisms and the 7-year risk of substantial cognitive decline adjusted for age, education level, center, depressive symptoms, comorbidity, anticholinergic medication, activity limitations, the *APOE ε4* allele and baseline cognitive performance (Table 2). Neither *ESR1* polymorphism was significantly associated with the risk of cognitive decline on any of the tests. There was only a weak trend for women homozygous for the alternative C allele of *rs2234693* to have a reduced risk of decline in executive function, compared to homozygous wild-type participants

Table 1 Baseline characteristics of women in the study ($n=3799$).

Baseline characteristic	Profile of cohort
	Median (Interquartile range)
Age (years)	73 (69-77)
	Frequency
Educational level (≥12 years of schooling)	24.8%
Moderate/severe activity limitations	7.7%
Use of anticholinergic medication	9.2%
Comorbidity[a]	46.7%
Depressive symptoms (CES-D ≥16)	28.1%
At least one ApoE-ε4 allele	19.9%
Recruitment left	
Bordeaux	19.8%
Dijon	57.4%
Montpellier	22.8%
	Median (Interquartile range)
Baseline cognitive test scores	
Mini-Mental State Examination (MMSE)	28 (26-29)
Isaacs Set Test	48 (41-55)
Benton's Visual Retention Test	12 (10-13)
Trail Making Test A, s	51 (41-65)
Trail Making Test B, s	99 (77-133)

[a]Includes cerebro- and cardio-vascular disease, more than one chronic illnesses (high blood pressure, high cholesterol, diabetes, thyroid problems, asthma), or cancer diagnosed within the last 2 years.

Table 2 The association between ESR polymorphisms and substantial cognitive decline over the 7-year follow-up (n=3799[a]).

Multivariate adjusted ± Cox proportional hazards analysis, HR (95% CI), p						
SNP and genotype	*N*	Global function MMSE decline ≥3[b]	Verbal fluency Isaacs decline ≥10[b]	Visual memory BVRT decline ≥3[b]	Psychomotor speed TMTA decline ≤11[c]	Executive function TMTB decline ≤57[c]
rs2234693						
TT	1147	1	1	1	1	1
CT	1900	1.13 (0.86-1.47), 0.36	1.06 (0.90-1.24), 0.46	0.96 (0.78-1.19), 0.74	0.93 (0.78-1.09), 0.36	0.99 (0.83-1.18), 0.89
CC	752	1.00 (0.71-1.40), 0.98	1.03 (0.84-1.26), 0.72	0.97 (0.74-1.26), 0.82	0.87 (0.70-1.07), 0.18	0.80 (0.63-1.01), 0.06
rs9340799						
AA	1576	1	1	1	1	1
GA	1767	1.01 (0.79-1.29), 0.89	1.03 (0.89-1.20), 0.61	0.97 (0.79-1.18), 0.78	1.02 (0.81-1.27), 0.88	1.01 (0.86-1.20), 0.88
GG	456	0.88 (0.60-1.30), 0.54	1.12 (0.89-1.39), 0.31	0.86 (0.64-1.17), 0.36	0.95 (0.67-1.35), 0.78	0.84 (0.64-1.10), 0.21
rs1256049						
GG	3490	1	1	1	1	1
GA/AA	309	0.89 (0.57-1.38), 0.62	1.14 (0.89-1.47), 0.29	**1.64 (1.23-2.18), 0.0007**	**1.43 (1.12-1.83), 0.004**	1.03 (0.77-1.39), 0.83
rs4986938						
GG	1397	1	1	1	1	1
AG	1845	0.86 (0.67-1.10), 0.24	0.93 (0.80-1.08), 0.35	0.92 (0.75-1.13), 0.44	**0.83 (0.71-0.97), 0.02**	1.16 (0.98-1.38), 0.09
AA	557	0.85 (0.60-1.21), 0.39	0.90 (0.72-1.12), 0.36	1.14 (0.87-1.50), 0.31	0.81 (0.65-1.02), 0.08	0.80 (0.61-1.04), 0.10
rs1275172						
GG	1230	1	1	1	1	1
TG	1915	1.10 (0.85-1.43), 0.44	1.01 (0.87-1.19), 0.83	0.96 (0.78-1.17), 0.78	1.16 (0.97-1.38), 0.09	1.16 (0.96-1.39), 0.12
TT	654	1.14 (0.81-1.61), 0.43	1.11 (0.91-1.36), 0.82	0.80 (0.59-1.07), 0.14	1.16 (0.94-1.45), 0.17	1.15 (0.91-1.45), 0.26

[a]Except in the case of TMTA and TMTB where n=3308.
[b]Models adjusted for age, education level (≥12 years of schooling), recruitment center, depressive symptoms, comorbidity, anticholinergic medication, activity limitations, the ApoE-ε4 allele and baseline cognitive performance.
[c]Full details are given in the methods.

(p=0.06). On the other hand, a number of significant associations were identified with *ESR2* polymorphisms. The A allele of *ESR2 rs1256049* was associated with a significantly increased risk of substantial decline on two of the cognitive tasks, increasing the risk of decline in visual memory by 64% and that of psychomotor speed by 43%. The former association was highly significant (p=0.0007) and remained so after Bonferroni adjustment. The *rs4986938* polymorphism was also associated with the risk of decline in psychomotor speed. This association reached uncorrected significance levels only in the comparison of AG vs. GG, where the former was associated with a reduced risk of decline, but there was a similar trend for women homozygous AA vs. GG. There was no evidence of effect modification from hormone treatment or *APOE-ε4*, as the corresponding interaction terms were non-significant in these models. All significant associations remained if the 117 participants with fluctuating cognitive scores were included in the analysis as decliners.

In the sub-sample of 3010 women without MCI at baseline, 28.5% were diagnosed with incident MCI over the 7-year follow-up. In multivariable-adjusted Cox proportional hazards models (Supplementary Table S2), the A allele of ESR2 *rs1256049* was again found to be associated with an increased risk of MCI (HR: 1.31), but this only reached traditional significance levels (p=0.02).

4. Discussion

To our knowledge this is the first study to prospectively examine associations between both *ESR1* and *ESR2* polymorphisms and different domains of cognitive function. In

this well characterized cohort of older postmenopausal women, we found significant associations between *ESR2* variants and the 7-year risk of substantial cognitive decline in visual memory and psychomotor speed, as well as the incidence of MCI. In terms of the *ESR1*, our hypothesis was only partly supported, as these *ESR1* variants were not significantly associated with decline on any of the cognitive tasks.

The largest previous study in this field evaluated the association between these same *ESR1* polymorphisms (*rs2234693* and *rs9340799*) and baseline MMSE scores among 6056 elderly (den Heijer et al., 2004) and these results support our finding of no association. Other studies which examined overall decline in MMSE performance provide some weak evidence of an association. Yaffe et al. found that among 2625 older women *rs2234693* TT, compared to CC, was associated with an increased risk of cognitive impairment, but they used a definition which incorporated not only a 3-point decline in modified MMSE scores, but also low follow-up scores, or a physician diagnosis of dementia (Yaffe et al., 2002). Cognitive impairment and dementia however, are quite heterogeneous conditions, with impairment in MMSE being a relatively poor predictor of later dementia (Ritchie et al., 2001) and thus different risk factors are likely to be involved. A more recent study which grouped the AG and AA genotypes of *rs9340799*, reported a borderline significant association with 4-year decline in modified MMSE scores among 1343 older women (Yaffe et al., 2009). Only one prior study, the SWAN study, has examined the potential for ESR variants to be associated with specific domains of cognitive performance. This was a relatively small cross-sectional study (n=875) with a racially mixed population of perimenopausal women (Kravitz et al., 2006). While no significant associations were found with performance on the tasks of working memory and perceptual speed, the GG genotype of *rs9340799* was associated with better immediate recall among African and Caucasian women. Caucasian women with *rs2234693* CC also performed significantly better on the delayed recall task. They did not examine executive function which was the only test in our study where *rs2234693* CC showed some evidence, although non-significant, of a beneficial effect (associated with reduced decline).

In terms of *ESR2* polymorphisms, our study suggests that the A allele of *rs1256049* is associated with an increased risk of cognitive decline in visual memory and psychomotor speed. It is not known how this particular variant could influence estrogen signaling and thus why it would be specifically associated with these cognitive domains, but it is likely to be in linkage disequilibrium with other yet unidentified functional variants. Interestingly prior analysis of cognitive function in this population indicated that poor baseline performance on specifically these two tests were associated with both reproductive factors which can influence lifetime endogenous estrogen exposure, as well as current use of HT (Ryan et al., 2009). Current HT use was also significantly associated with 4-year decline in psychomotor speed. Furthermore, other studies have also reported positive effects of estrogen treatment on these cognitive domains (Dumas et al., 2006; Resnick et al., 2006).

The *rs1256049* polymorphism has previously being investigated in a study of 4-year decline in modified MMSE scores, and like our findings for MMSE, they found no significant association (Yaffe et al., 2009). This may be due to the insensitivity of the MMSE, a dementia screening test, to detect milder forms of cognitive change. The only other study to examine ESR2 variants, the SWAN study of 875 women (as mentioned above) also examined *rs1256049*, and two other ESR2 variants. They failed to find any significant associations with baseline performance on tasks assessing word recall, working memory or perceptual speed (Kravitz et al., 2006).

Limitations to this study should be considered when interpreting the results. This includes bias from the exclusion of some participants initially recruited to the 3C study, and given that only a selected number of gene variants were examined, other variants not investigated here may also be associated with cognitive decline. The number of associations in this study may have increased the risk of a type 1 error, however the principal finding remained significant even after Bonferroni correction. Furthermore, the comparisons are not truly independent (both ESR1 polymorphisms and all ESR2 polymorphisms are in high linkage disequilibrium) and some consistency was observed across cognitive tests. Population stratification could not be controlled for as French law prohibits the collection of data related to ethnicity, but the genotype frequencies were similar to those previously observed in white Europeans (Domingues-Montanari et al., 2008; Ioannidis et al., 2002).

Strengths of this analysis relate to the 3C study design which followed a large population of community-based and well-characterized women prospectively over 7 years. Different cognitive tasks were assessed at baseline and three times over follow-up, and incident MCI was also examined. Potential confounding has been minimized by including a large range of health and lifestyle factors, notably those which have previously been associated with cognitive function in the elderly.

The association between estrogen and later-life cognition remains complex, but our large prospective study suggests that *ESR2* variants may be preferentially associated with decline on certain cognitive tasks. These tasks could thus be most susceptible to changes in estrogen signaling. We found no strong evidence for an association between common *ESR1* polymorphisms and the risk of cognitive decline. These findings require further confirmation in other large prospective cohorts investigating a larger range of domain specific cognitive tasks.

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Contributors

J. Ryan generated the working hypotheses for this study in collaboration with M.-L. Ancelin, performed the statistical analysis and wrote the manuscript. I. Carrière supervised analysis and assisted with manuscript revision. H. Amieva, O. Rouaud and C. Berr were responsible for data management within their respective center and assisted with manuscript revision; K. Ritchie is the chief investigator of the Montpellier center for the 3C study and assisted with manuscript revision. P.-Y. Scarabin was responsible for the ESR genotyping and assisted with manuscript revision. M.-L. Ancelin designed the study, and had a major role in reviewing the paper. All authors have approved the final version of the manuscript.

Conflict of interest

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 declare that they have no conflicts of interest.

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Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.euroneuro.2013.06.003.

References

Ancelin, M.L., Carriere, I., Barberger-Gateau, P., Auriacombe, S., Rouaud, O., Fourlanos, S., Berr, C., Dupuy, A.M., Ritchie, K., 2012. Lipid lowering agents, cognitive decline, and dementia: the three-city study. J. Alzheimer's. Dis. 30, 629–637.

Ancelin, M.L., Ritchie, K., 2005. Lifelong endocrine fluctuations and related cognitive disorders. Curr. Pharm. Des. 11, 4229–4252.

Artero, S., Petersen, R., Touchon, J., Ritchie, K., 2006. Revised criteria for mild cognitive impairment: validation within a longitudinal population study. Dement. Geriatr. Cogn. Disord. 22, 465–470.

Brann, D.W., Dhandapani, K., Wakade, C., Mahesh, V.B., Khan, M.M., 2007. Neurotrophic and neuroprotective actions of estrogen: basic mechanisms and clinical implications. Steroids 72, 381–405.

Craig, M.C., Murphy, D.G., 2007. Oestrogen, cognition and the maturing female brain. J. Neuroendocrinol. 19, 1–6.

den Heijer, T., Schuit, S.C., Pols, H.A., van Meurs, J.B., Hofman, A., Koudstaal, P.J., van Duijn, C.M., Uitterlinden, A.G., Breteler, M.M., 2004. Variations in estrogen receptor alpha gene and risk of dementia, and brain volumes on MRI. Mol. Psychiatry 9, 1129–1135.

Domingues-Montanari, S., Subirana, I., Tomas, M., Marrugat, J., Senti, M., 2008. Association between ESR2 genetic variants and risk of myocardial infarction. Clin. Chem. 54, 1183–1189.

Dumas, J., Hancur-Bucci, C., Naylor, M., Sites, C., Newhouse, P., 2006. Estrogen treatment effects on anticholinergic-induced cognitive dysfunction in normal postmenopausal women. Neuropsychopharmacology 31, 2065–2078.

Henderson, V.W., 2010. Action of estrogens in the aging brain: dementia and cognitive aging. Biochim. Biophys. Acta 1800, 1077–1083.

Hojo, Y., Murakami, G., Mukai, H., Higo, S., Hatanaka, Y., Ogiue-Ikeda, M., Ishii, H., Kimoto, T., Kawato, S., 2008. Estrogen synthesis in the brain—role in synaptic plasticity and memory. Mol. Cell. Endocrinol. 290, 31–43.

Ioannidis, J.P., Stavrou, I., Trikalinos, T.A., Zois, C., Brandi, M.L., Gennari, L., Albagha, O., Ralston, S.H., Tsatsoulis, A., 2002. 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. 17, 2048–2060.

Kravitz, H.M., Meyer, P.M., Seeman, T.E., Greendale, G.A., Sowers, M.R., 2006. Cognitive functioning and sex steroid hormone gene polymorphisms in women at midlife. Am. J. Med. 119, S94–S102.

Maruyama, H., Toji, H., Harrington, C.R., Sasaki, K., Izumi, Y., Ohnuma, T., Arai, H., Yasuda, M., Tanaka, C., Emson, P.C., Nakamura, S., Kawakami, H., 2000. Lack of an association of estrogen receptor alpha gene polymorphisms and transcriptional activity with Alzheimer disease. Arch. Neurol. 57, 236–240.

Olsen, L., Rasmussen, H.B., Hansen, T., Bagger, Y.Z., Tanko, L.B., Qin, G., Christiansen, C., Werge, T., 2006. Estrogen receptor alpha and risk for cognitive impairment in postmenopausal women. Psychiatr. Genet. 16, 85–88.

Osterlund, M.K., Hurd, Y.L., 2001. Estrogen receptors in the human forebrain and the relation to neuropsychiatric disorders. Prog. Neurobiol. 64, 251–267.

Radloff, L., 1977. The CES-D scale: a self-report depression scale for research in the general population. Appl. Psychol. Meas. 1, 385–401.

Resnick, S.M., Maki, P.M., Rapp, S.R., Espeland, M.A., Brunner, R., Coker, L.H., Granek, I.A., Hogan, P., Ockene, J.K., Shumaker, S.A., 2006. Effects of combination estrogen plus progestin hormone treatment on cognition and affect. J. Clin. Endocrinol. Metab. 91, 1802–1810.

Ritchie, K., Artero, S., Touchon, J., 2001. Classification criteria for mild cognitive impairment: a population-based validation study. Neurology 56, 37–42.

Ryan, J., Carriere, I., Scali, J., Ritchie, K., Ancelin, M.L., 2009. Life-time estrogen exposure and cognitive functioning in later life. Psychoneuroendocrinology 34, 287–298.

Ryan, J., Scali, J., Carriere, I., Peres, K., Rouaud, O., Scarabin, P.Y., Ritchie, K., Ancelin, M.L., 2011. Oestrogen receptor polymorphisms and late-life depression. Br. J. Psychiatry 199, 126–131.

The 3C Study Group, 2003C. Vascular factors and risk of dementia: design of the three city study and baseline characteristics of the study population. Neuroepidemiology 22, 316–325.

Thiebaut, A.C., Benichou, J., 2004. Choice of time-scale in Cox's model analysis of epidemiologic cohort data: a simulation study. Stat. Med. 23, 3803–3820.

Wilson, R.S., Boyle, P.A., Segawa, E., Yu, L., Begeny, C.T., Anagnos, S.E., Bennett, D.A., 2013. The influence of cognitive decline on well-being in old age. Psychol. Aging 28, 304–313.

Yaffe, K., Lindquist, K., Sen, S., Cauley, J., Ferrell, R., Penninx, B., Harris, T., Li, R., Cummings, S.R., 2009. Estrogen receptor genotype and risk of cognitive impairment in elders: findings from the Health ABC study. Neurobiol. Aging 30, 607–614.

Yaffe, K., Lui, L.Y., Grady, D., Stone, K., Morin, P., 2002. Estrogen receptor 1 polymorphisms and risk of cognitive impairment in older women. Biol. Psychiatry 51, 677–682.

Zhao, L., Wu, T.W., Brinton, R.D., 2004. Estrogen receptor subtypes alpha and beta contribute to neuroprotection and increased Bcl-2 expression in primary hippocampal neurons. Brain Res. 1010, 22–34.