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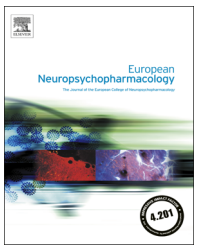

SHORT COMMUNICATION

# 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