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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 (AD), but this association could be modified by genetic and environmental factors.

Methods: The association between five *ESR* α (*ESR1*) and β (*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 specifically associated with a small increased risk of AD (adjusted hazard ratio [HR]: 1.54, 95% confidence interval [CI]: 1.03–2.28, $P = .03$). 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 with 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* polymorphisms on the risk of all dementias ($P = .04$). Hormone treatment did not modify these associations, and there were no significant associations in men.

Conclusions: Although 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.

Keywords: Alzheimer's disease; Dementia; Estrogen receptor polymorphisms; ESR1; ESR2; Gender-specific; *APOE*; Epidemiology; Cohort study

1. Background

Dementia is a multifactorial disease that has been associated with many 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.

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Epidemiological studies have reported that women have a higher incidence of dementia, and in particular AD, compared with men [4], which suggests the potential involvement of steroid hormones such as estrogen. In support of this, estrogen is synthesized in the brain by the aromatization of androgens, with estrogen receptors (ESRs) 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, several 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 randomized controlled trial (see for review [7]). It is plausible that genetic variants that modify estrogen signaling, such as polymorphisms in the estrogen receptors α (*ESR1*) and β (*ESR2*), could be candidate risk genes for dementia.

Indeed, several 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]; however, 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 (odds ratio [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. However, there are limitations to case-control studies, 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. On the basis of 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 (3C) Study is a multicenter 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 standardized 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. Thus, these data are based on 6959 men and women. Compared with the analyzed sample, participants not included in this analysis were more likely to be older and have a lower education level, physical incapacities, depressive symptoms, and comorbidity (P values $<.005$) at baseline, and they were more likely to be diagnosed with dementia during the follow-up period ($P < .001$). 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 *rs4986938*, in which excluded participants were more likely to carry the variant A allele ($P = .02$).

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, when possible, magnetic resonance images or computed tomography scans were also collected. A neurologist then examined all participants suspected of having dementia. 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 the *Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV),* revised criteria and etiology. AD 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/). On the basis of 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 polymerase chain reaction (PCR) single-nucleotide polymorphism (SNP) genotyping system (KASPar). The amplified PCR products were analyzed 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, because 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 that 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 $\geq$7.0 mmol/L or reported treatment), hypercholesterolemia (total cholesterol $\geq$6.2 mmol/L), hypertension (resting blood pressure $\geq$160/95 mmHg or treated), thyroid problems, or cancer diagnosis within the last 2 years.

2.5. Statistical analysis

χ^2 tests were used to compare the distribution of *ESR* genotypes with those predicted under the Hardy-Weinberg equilibrium, and pairwise linkage disequilibrium was estimated. Baseline characteristics of participants associated with dementia incidence were identified using χ^2 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 nonproportionality 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 those that 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 because 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., Cary, NC).

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. Approximately 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 with nondemented 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 with 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* ($\chi^2_1 = 4.3$, $P = .04$). 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 specifically found between *rs2234693* and the risk of AD, with the CC genotyping being associated with a 1.5 times increased risk (95% confidence interval [CI]: 1.03–2.28). However, after correction for multiple testing ($P < .007$), this association would not remain significant. There was a weak nonsignificant 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 allele by including an interaction term in the models ($P = .068$), and subsequent stratified analyses indicated that the CC genotype of *rs2234693* further increased the risk of AD

Table 1
Baseline characteristics of participants according to the incidence of dementia

Baseline characteristic	7-year incident dementia: No ($n = 6488$)	7-year incident dementia: Yes ($n = 471$)	Test for difference
	Mean (SD)		*P*
Age (years)	73.6 (5.2)	77.3 (5.4)	<.0001
BMI (kg/m^2)	25.7 (3.9)	25.7 (4.3)	.73
	%		*P*
Gender			
Female	61.2	61.1	.99
Male	38.8	38.9	
Center			
Bordeaux	20.9	28.7	<.0001
Dijon	55.3	54.8	
Montpellier	23.8	16.5	
≥12 years of schooling	30.1	25.5	.03
Current heavy drinker*	16.0	15.8	.91
Heavy smoker (10 pack years)	37.8	35.3	.28
Moderate/severe activity limitations	6.6	17.4	<.0001
Use of anticholinergics	7.2	13.2	<.0001
Comorbidity†	47.9	57.1	.0001
Depressive symptoms‡	22.4	31.0	<.0001
At least one *APOE*-ε4 allele	19.4	27.8	<.0001
Current use of hormone treatment§	15.5	5.5	<.0001

*Defined as ≥24 g of alcohol each day.

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

‡Assessed using the CES-D (CES-D ≥ 16).

§Percentage based on 3968 women without and 288 women with incident dementia.

Table 2
Adjusted Cox proportional hazards analysis for the association between *ESR2* polymorphisms and 7-year incidence of dementia in men and women

SNP and genotype	*N*	Men ($n = 2703$): All-cause dementia HR (95% CI), *P*	Men ($n = 2703$): AD HR (95% CI), *P*
rs1256049			
GG	2484	1	1
GA/AA	219	1.01 (0.60–1.72), $P = .96$	0.99 (0.50–1.96), $P = .97$
rs1275172			
GG	874	1	1
TG	1331	0.77 (0.56–1.06), $P = .11$	0.72 (0.48–1.06), $P = .10$
TT	498	0.83 (0.68–1.16), $P = .37$	0.66 (0.38–1.14), $P = .13$
rs4986938			
GG	1020	1	1
AG	1249	0.92 (0.66–1.27), $P = .61$	1.05 (0.70–1.58), $P = .82$
AA	434	1.18 (0.78–1.71), $P = .43$	1.30 (0.80–1.53), $P = .32$
		Women ($n = 4256$)	
SNP and genotype	N	All-cause dementia HR (95% CI), *P*	AD HR (95% CI), *P*
rs1256049			
GG	3904	1	1
GA/AA	352	1.34 (0.91–1.99), $P = .14$	1.09 (0.65–1.83), $P = .73$
rs1275172			
GG	1375	1	1
TG	2157	1.04 (0.80–1.36), $P = .75$	0.94 (0.69–1.29), $P = .70$
TT	724	0.95 (0.67–1.36), $P = .78$	0.91 (0.60–1.37), $P = .64$
rs4986938			
GG	1565	1	1
AG	2071	0.98 (0.76–1.26), $P = .86$	0.93 (0.68–1.25), $P = .62$
AA	620	0.86 (0.75–1.30), $P = .42$	0.99 (0.66–1.52), $P = .99$

NOTE. Adjusted for age, education level, recruitment center, activity limitations, comorbidity, depressive symptoms, the use of anticholinergic medication, and *APOE* ε4 allele.

Table 3
Adjusted Cox proportional hazards analysis for the association between *ESR1* polymorphisms and 7-year incidence of dementia in men and women

		Men (n = 2703)	
		All-cause dementia	AD
SNP and genotype	N	HR (95% CI), P	HR (95% CI), P
rs2234693			
TT	812	1	1
CT	1362	0.85 (0.61–1.18), P = .33	0.85 (0.57-1.28), P = .44
CC	529	0.73 (0.47–1.13), P = .16	0.72 (0.42-1.29), P = .23
rs9340799			
AA	1138	1	1
GA	1234	0.84 (0.62–1.15), P = .27	0.84 (0.57-1.23), P = .37
GG	331	0.65 (0.39–1.08), P = .10	0.58 (0.30-1.11), P = .10
		Women (n = 4256)	
		All-cause dementia	AD
SNP and genotype	N	HR (95% CI), P	HR (95% CI), P
rs2234693			
TT	1289	1	1
CT	2137	1.21 (0.92–1.59), P = .18	1.31 (0.94-1.84), P = .11
CC	830	1.23 (0.88–1.72), P = .24	1.54 (1.03-2.28), P = .03
rs9340799			
AA	1770	1	1
GA	1987	1.22 (0.95–1.57), P = .12	1.29 (0.95-1.74), P = .11
GG	499	1.23 (0.84–1.80), P = .28	1.49 (0.97-2.29), P = .06

NOTE. Adjusted for age, education level, recruitment center, activity limitations, comorbidity, depressive symptoms, use of anticholinergic medication, and *APOE* ε4 allele.

associated with the *APOE* ε4 allele (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, $P < .0001$). However, when stratified by *rs2234693*, 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 allele ($P < .0001$). For women with the TT genotype, *APOE*-ε4 was associated with a 1.9-fold increased risk that just failed to reach significance (P = .06), and for women with the CT genotype there was no significantly increased risk of AD associated with the *APOE* ε4 allele (P = .12). Further adjustment for BMI or the current use of hormone treatment 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.

On the basis of 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 we found a nominally significant interaction with *rs1256049* on the risk of dementia (P = .04). Indeed, although *rs1256049* was not associated with dementia risk across all women (hazard ratio [HR]: 1.34, P = .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 = .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 the *ESR2 rs1256049* GG and *ESR1 rs2234693* C allele or the *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.

Table 4
The 7-year risk of AD associated with the *APOE* ε4 allele in adjusted Cox proportional hazards analysis in women, stratified by *ESR1 rs2234693*

	AD, N (cases/total)		
	rs2234693 TT (50 of 1261)	*rs2234693* CT (104 of 2091)	*rs2234693* CC (49 of 819)
APOE ε4			
Noncarrier	1	1	1
At least 1 allele	1.94 (0.99–3.80), P = .06	1.45 (0.91–2.32), P = .12	3.24 (1.81–5.79), $P < .0001$

NOTE. Adjusted for age, education level, recruitment center, activity limitations, comorbidity, depressive symptoms, and the use of anticholinergic medication.

Table 5
Adjusted Cox proportional hazards analysis for the 7-year incidence of dementia associated with the *ESR2 rs1256049* A allele in women, stratified by *rs2234693*

	All-cause dementia, N (cases/total)		
	rs2234693 TT (78 of 1289)	*rs2234693* CT (150 of 2137)	*rs2234693* CC (60 of 830)
rs1256049			
GG	1	1	1
GA/AA	2.66 (1.38–5.11), P = .003	1.19 (0.69–2.03), P = .53	0.61 (0.19–1.98), P = .49

NOTE. Adjusted for age, education level, recruitment center, activity limitations, comorbidity, depressive symptoms, the use of anticholinergic medication, and *APOE* ε4 allele.

Several 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 7 years and examining the association between *ESR1* and *ESR2* polymorphisms and the incidence of all-cause dementia or AD. On the basis of our sample size, the incidence of all-cause dementia (6.8%), and the frequency of the *ESR* genotypes, our study could detect a 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 most 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 because most significant findings have been found in Japanese populations [10,11,37]. Although these *ESR1* genotype frequencies are relatively similar between Caucasian and Asian populations, ethnic differences in linkage disequilibrium between these polymorphisms and other functional polymorphisms are 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 because 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 specifically influence the pathogenic processes of AD. Furthermore, the association between *ESR1* polymorphisms and AD was gender-specific, with a significant association found only in women. In men, the HR for the female "risk genotypes" was actually below 1.0, indicating a nonsignificant 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 with men, and this 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 that 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*. However, the only other study to examine non-AD dementia found that the A allele of *rs4986938* was more frequent in women with vascular dementia ($n = 60$) compared with 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 ($P = .04$ and .03, respectively) [21]. Unfortunately, because of 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 ($P = .04$). 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, $P = .003$). Such an interaction between the two estrogen receptor subtypes could have a biological sense, although the functional significance of these particular polymorphisms remains unknown. In vitro studies have shown that *ESR* transcriptional activation and the response to estrogens are 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 less than .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 ($P = .068$). Across all women, the *APOE* ε4 allele was associated with a highly significant 1.9 times increased risk ($P < .0001$); however, in women with the CC genotype, the risk of AD with the *APOE*-ε4 allele increased to 3.2-fold ($P < .0001$). This observation is supported by findings from two case-control studies [8,9] that grouped participants on the basis of 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 frequent in AD patients with the *APOE* ε4 than in those without [11]. However, previous case-control studies that have directly tested for an *ESR1* × *APOE* ε4 interaction have failed to find a significant association ($P > .10$) [15,16,21,43]. However, it is important to note that most of these null findings have accompanied studies that 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 β-amyloid accumulation, which could be reversed by estrogen treatment [47]. Estrogen has also been shown to increase *APOE* mRNA levels and thus upregulate expression of the *APOE* gene [48], which occurred in large part through *ESR1* [48]. Previous research from epidemiological studies also suggests an interaction between estrogen and *APOE* because 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 nondemented 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 few 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 many 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 sociodemographic, health, and lifestyle variables, and our study was adequately powered to detect moderate effect sizes. This contrasts with previous studies that have predominantly presented only age-matched analyses in small populations and have failed to consider potentially important confounding factors such as 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 several 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]. Therefore, large-scale meta-analyses that combine results from multiple studies, such as the Alzheimer Research Forum [17] (http://www.alzgene.org), are 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*s, most previous association studies have been undertaken using small, specific populations within a case-control design, which has obvious limitations and thus requires conformation. Our multicentric prospective study of almost 7000 participants has enabled us to examine for the first time the gender-specific association between *ESR1* and *ESR2* polymorphisms and 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 that is 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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RESEARCH IN CONTEXT

1. Systematic review: A literature search from 1980 to March 2012 was conducted using the MEDLINE, Web of Knowledge, and PsychINFO databases and combining the terms "dementia" or "Alzheimer's disease" and "estrogen receptor polymorphisms" or "estrogen receptor variants". Studies listed on the Alzheimer's Research Forum (http://www.alzgene.org) were also examined.
2. Interpretation: This is the largest prospective study to investigate the association between incident dementia and *ESR1* polymorphisms and the first prospective study to examine *ESR2* polymorphisms. Analyses have taken into account gender, *APOE* ε4, comorbidity, and hormone treatment. Results suggest a weak association with *ESR1 rs2334693*, specific for AD and in women only, but this polymorphism may act as an effect modifier.
3. Future directions: *ESR* polymorphisms do not appear to be strongly associated with dementia in Caucasians, but the findings that *rs2334693* may modify the risk of AD associated with *APOE* ε4 and a significant *ESR1-ESR2* interaction requires replication in another large population-based prospective study.

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