Effect of interactions between *APOE* and *ESR1* polymorphisms on cognitive functions in postmenopausal women

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Submitted: 9 September 2017
Accepted: 13 November 2017

Arch Med Sci 2021; 17 (1): 31–39
DOI: https://doi.org/10.5114/aoms.2018.72972
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Abstract

Introduction: During menopause the level of estrogens is decreased, which may lead to cognitive impairment or dementia. Some forms of genetic polymorphism were found to be related to cognitive functions, including *APOE* and *ESR1* (*Pvu*II and *Xba*I) polymorphisms. In the present study we aimed to analyze the impact of interactions between *APOE* and *ESR1* polymorphisms on cognitive functions in the group of postmenopausal women.

Material and methods: The study group consisted of 266 postmenopausal women aged 50–65 years without symptoms of dementia. A computerized battery of the Central Nervous System Vital Signs (CNS VS) test was used to diagnose cognitive functions. *APOE* and *ESR1* polymorphisms were genotyped using multiplex PCR and PCR-RFLP methods, respectively. Statistical analysis was performed using two-way analysis of variance in Statistica software.

Results: The best memory, visual memory, processing and psychomotor speeds were found in women carrying the C allele of the *Pvu*II polymorphism (TC + CC genotypes) in the presence of the *APOE* ε2/ε3 genotype, while a lower outcome was noted in women with ε3/ε3, and the lowest if they had the ε4 allele. In the case of women with TT genotype of the *Pvu*II polymorphism, cognitive functioning did not decrease in women with the ε4 allele. A similar effect on cognitive functions was observed for AG + GG genotypes of the *Xba*I and *APOE* polymorphisms. Women who simultaneously carried CC *Pvu*II and GG *Xba*I genotypes had the lowest cognitive functions.

Conclusions: Interactions of polymorphic variants of *APOE* and *ESR1* genes influenced cognitive functions in postmenopausal women.

Key words: apolipoprotein E, estrogen receptor alpha, cognition, postmenopause.

Introduction

Estrogens exert a pleiotropic effect on a woman's whole body: the skin, adipose tissue, bones, the central nervous system, cardiovascular and di-

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gestive systems, not only on the reproductive system [1, 2]. Previous reports identified the role of estrogens in the regulation of synaptogenesis in the region of the dorsal hippocampus in female rats [3]. The naturally occurring low levels of estrogens noted before and after menopause may be related to cognitive impairments or dementia [4]. Hence, hormone replacement therapy (HRT) can reduce the risk of dementia in postmenopausal women, as confirmed by the meta-analysis of LeBlanc *et al.* [5].

Estrogen interacts with two estrogen receptors: estrogen receptor-α (ERα) and estrogen receptor-β (ERβ), encoded by *ESR1* and *ESR2* genes, respectively. Two polymorphisms within *ESR1* (rs9340799 – 351A>G (*Xba*I) and rs2234693 – 397T>C (*Pvu*II), separated by only 46 base pairs) are noted to play a role in several diseases [6–8]. It is also suggested that both *ESR1* polymorphisms may have an impact on cognitive functioning [9, 10]. However, there are also contrary results in the topic [11].

Available data establish a clear relationship between estrogens and apolipoprotein E (APOE). Earlier results also showed that the *APOE* ε4 allele was related to a range of disorders, including lipid profile [12], Alzheimer disease (AD) [13, 14], and coronary artery disease, often in the presence of other candidate polymorphisms [15]. It was also observed that healthy individuals carrying the *APOE* ε4 allele had significantly worse overall verbal episodic memory than healthy non-carriers [16]. A study on mouse cortical neurons showed that estradiol influenced neurite growth through an ApoE-dependent mechanism; therefore, HRT may have a different impact on chronic neurological diseases due to the presence of a particular *APOE* genotype [17].

The aim of the present study was to analyze the impact of interactions between *APOE* and *ESR1* polymorphisms on cognitive functioning in postmenopausal women.

Material and methods

Study group

The study was conducted in 2014 at the Institute of Rural Health in Lublin, Poland. The study group comprised 266 women from south-eastern Poland. The inclusion criteria were: age 50–65 years, with a minimum period of 2 years since the last menstrual period. The exclusion criteria were: any chronic diseases within the last 5 years, medical history of mental diseases, addiction to drugs and/or alcohol, diagnosed disease entity with the symptoms of dementia, current or past use of HRT, severe menopausal symptoms according to the Kupperman menopausal index [18]. A brief Montreal Cognitive Assessment (MoCA) test was performed in order to exclude women who presented with features of dementia. Only women who obtained scores of at least 26 were included in the study.

Computerized neurocognitive assessment software for CNS-Vital Signs

Assessment of cognitive functions [19] was performed based on the diagnostic equipment, CNS Vital Signs (1829 East Franklin Street, Bldg 500, Chapel Hill NC 27514, 919-933-0932). The instrument, consisting of a battery of computer tests, is standardized, and was subjected to a full validation procedure. It has many cultural and language adaptations, including one for Polish. Nine cognitive functions – Memory, Verbal Memory, Visual Memory, Processing Speed, Executive Functioning, Psychomotor Speed, Reaction Time, Complex Attention, Cognitive Flexibility – were assessed. The CNS VS test provides the Neurocognitive Index based on five cognitive functions: memory, psychomotor speed, reaction time, attention, and cognitive flexibility. Standard scores of the NCI and of nine cognitive functions were analyzed and interpreted as: above average (> 109), average (90–109), low average (80–89), low (70–79), very low (< 70), with higher values of standard scores indicating better cognitive functions.

DNA isolation

Genomic DNA isolation was derived from 0.2 ml of human blood by the QIAamp DNA Blood Mini Kit (Qiagen, USA), as per the producer's instructions. The amount and purity of the extracted DNA were measured using the NanoDrop spectrophotometer.

APOE polymorphism

Multiplex polymerase chain reaction (PCR) was carried out according to Yang *et al.* [20], with some modifications. PCR reactions were made in a single reaction tube with six primers, including two common primers and two specific primers for each of two single nucleotide polymorphism (SNP) sites. The multiplex PCR reaction was carried out in a 50 µl reaction volume containing the following mix of reagents: 1.25 U *Taq* DNA polymerase, 1× PCR buffer containing 15 mM $MgCl_2$ and 1× Q buffer (all from Qiagen, USA), 0.2 mM each of dNTP (Fermentas, Lithuania), 0.5 µM of each of six primers: FO, RO, FI-1, RI-1, FI-2, RI-2 (Eurogentec, Seraing, Belgium), nuclease-free water (Applied Biosystems, USA) and 5 µl of DNA. The reaction was performed in a C1000 Thermal Cycler (Bio-Rad) under the following conditions: initial denaturation at 95°C for 5 min, then 35 cycles (denaturation 95°C for 30 s, annealing at 60°C for 30 s, elongation at 72°C for 60 s); the final extension

step is at 72°C for 7 min. The reaction products were detected in 2.5% agarose gels in the standard electrophoresis conditions. After ethidium bromide staining, the strips were read under UV light. The size of the amplified DNA fragment, using two common outer primers (FO and RO), was 514 bp. Obtained DNA amplicons flanked by each of two sets of allele-specific inner primers (FI-1/RI-1 and FI-2/RI-2) showed different types of polymorphisms: 444 bp, 307 bp and 115 bp for ε3/ε4; 307 bp and 115 bp for ε3/ε3; 444 bp and 307 bp for ε4/ε4; 307 bp, 253 bp and 115 bp for ε2/ε3; 444 bp, 307 bp, 253 bp and 115 bp for ε2/ε4.

ESR1 polymorphisms

Polymorphisms of *ESR1* were determined using the restriction fragment length polymorphism (RFLP-PCR) method. PCR reaction was performed in a total amount of 50 µl containing: 1 U (1 µl) of DNA polymerase (Biotools), 1 PCR buffer (5 µl) containing 15 mM $MgCl_2$ (Biotools), 2.5 µl 2 mM dNTPs (final concentration 0.1 mM) (Fermentas, Vilnius, Lithuania), 1 µl of 10 µM of each of the two primers, 34.5 µl nuclease-free water (Applied Biosystems Inc., USA) and 5 µl of genomic DNA. The reactions were performed in a C1000 Thermal Cycler (Bio-Rad) and consisted of the initial denaturation (3 min at 95°C) and 30 cycles, each of which included the proper denaturation (30 s at 95°C), primer annealing (50 s at 62°C), elongation (50 s at 72°C), and the final elongation (7 min at 72°C). Electrophoresis was performed in 2% agarose gel in standard conditions. The products of PCR (1372 bp) were digested overnight at 37°C using 2 separate restriction enzymes for determining the polymorphisms: *Pvu*II (c.454-397 T>C) and *Xba*I (c.454-351 A>G). The products of restriction were electrophoresed in 2.5% agarose gel.

The alleles of the *Xba*I polymorphism were defined as A and G: heterozygote AG (fragments: 1372 bp, 936 bp, and 436 bp), homozygote GG (fragment: 1372 bp), and homozygote AA (fragments: 936 bp and 436 bp). The alleles of the *Pvu*II polymorphism were defined as T and C: heterozygote TC (fragments: 1372 bp, 982 bp, and 390 bp), homozygote TT (982 bp and 930 bp), and homozygote CC (1372 bp).

Statistical analysis

The data were statistically analyzed using Statistica software. We estimated mean values (M) with standard deviations (SD) for continuous variables, and absolute (*n*) and relative numbers (%) of occurrence of items for categorical variables. Two-way analysis of variance was used to compare cognitive functions versus *APOE* and *ESR1* polymorphisms. F statistics were used to test three different hypotheses: *APOE* polymorphism affects cognitive functions; *ESR1* polymorphism affects cognitive functions; and the interaction between *APOE* and *ESR1* polymorphisms affects cognitive functions. Due to the small sample sizes of women with ε4/ε4 and women with ε3/ε4, they were combined together for statistical analysis. One-way analysis of variance was used to compare cognitive functions versus interaction between *XbaI* and *PvuII* of *ESR1* polymorphisms due to the very small sample sizes or even empty cells in cross-sections.

The value of $p \leq 0.05$ was considered to indicate a significant difference.

Informed consent for participation in the study was obtained from all women. The study was approved by the Ethics Committee of the Institute of Rural Medicine in Lublin, Poland.

Results

Study group characteristics

A total of 266 postmenopausal women, aged 50-65 years, with an average age of 56.6 ±3.4 years, of mostly secondary education, were examined in the study. The majority of them were carriers of ε3/ε3 *APOE* polymorphism, with approximately half of them possessing AG genotype of the *ESR1 Xba*I polymorphism and a little fewer having TC genotype of the *ESR1 Pvu*II polymorphism (Table I).

Cognitive functions vs. *APOE* polymorphism

NCI and five of nine cognitive functions – executive functioning, psychomotor speed, reaction time, complex attention and cognitive flexibility – depended on *APOE* gene polymorphism (Table II). NCI was assessed as average in women with ε3/ε3 genotype, as low average in women with ε3/ε3, as low in women with ε3/ε4, and very low in women with ε4/ε4. Executive functioning and cognitive flexibility were assessed as average in women with ε3/ε3 genotype, as low in women with ε3/ε3 and ε3/ε4, and very low in women with ε4/ε4. Psychomotor speed was assessed as average in women with ε3/ε3 genotype, as low in women with ε3/ε3, and very low in women with ε3/ε4 and ε4/ε4. Reaction time was assessed as average in women with ε3/ε3 genotype, and as low average in women with ε3/ε3, ε3/ε4 and ε4/ε4. Complex attention was assessed as average in women with ε3/ε3 genotype, as low in women with ε3/ε3, and very low in women with ε3/ε4 and ε4/ε4.

Cognitive functions vs. *ESR1* polymorphisms

Three cognitive functions – processing speed, executive functioning and cognitive flexibility – depended on *ESR1 Pvu*II polymorphism (Table III).

Table I. Study group characteristics

Variable	Category	Parameter	Estimate
Age	Years	Min.–max., mean ± SD	50–65, 56.4 ±3.4
Level of education	Primary	*n* (%)	10 (3.76)
	Basic vocational	*n* (%)	23 (8.65)
	Secondary	*n* (%)	128 (48.12)
	Tertiary	*n* (%)	105 (39.47)
APOE polymorphism	ε2/ε3	*n* (%)	53 (19.92)
	ε3/ε3	*n* (%)	157 (59.02)
	ε3/ε4	*n* (%)	39 (14.66)
	ε4/ε4	*n* (%)	17 (6.39)
*ESR1 Pvu*II polymorphism	TT	*n* (%)	75 (28.20)
	TC	*n* (%)	119 (44.74)
	CC	*n* (%)	72 (27.07)
*ESR1 Xba*I polymorphism	AA	*n* (%)	92 (34.59)
	AG	*n* (%)	129 (48.50)
	GG	*n* (%)	45 (16.92)

Table II. Cognitive functions vs. *APOE* polymorphism

Cognitive function	Total		APOE								Comparisons between *APOE* genotypes	
			ε2/ε3		ε3/ε3		ε3/ε4		ε4/ε4			
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*F*	*P*-value
NCI	83.2	17.4	92.7	14.7	83.2	14.4	76.4	22.6	68.8	21.4	12.653	< 0.001
Memory	88.0	16.9	91.5	15.9	87.6	15.6	84.7	21.5	88.2	18.7	1.289	0.279
Verbal memory	89.5	19.2	93.7	19.6	89.5	18.1	83.4	23.5	91.1	14.4	2.225	0.086
Visual memory	91.9	15.5	93.3	11.6	91.4	15.7	92.9	16.6	90.2	21.4	0.333	0.802
Processing speed	79.3	14.6	82.2	15.0	78.9	15.0	77.4	13.2	79.2	12.6	0.942	0.421
Executive functioning	78.4	25.8	93.2	19.5	78.4	24.0	69.7	27.5	52.3	27.7	15.160	< 0.001
Psychomotor speed	82.9	18.3	91.5	13.9	82.3	16.1	76.3	25.3	76.1	21.9	6.894	< 0.001
Reaction time	87.5	16.3	93.8	20.0	85.4	14.2	87.5	15.2	87.1	19.8	3.663	0.013
Complex attention	81.0	28.6	95.9	19.9	81.9	26.1	67.9	33.8	55.7	32.6	13.757	< 0.001
Cognitive flexibility	77.1	27.0	92.4	20.9	77.3	24.9	66.0	30.5	53.1	27.6	14.133	<0.001

Processing speed was better in women who were TT homozygotes than in women with TC and CC genotypes. Executive functioning and cognitive flexibility were lower in women with TT genotype than in those with TC and CC genotypes.

Four cognitive functions – memory, verbal memory, visual memory and processing speed – depended on *ESR1 Xba*I (Table IV). Women with AA and AG genotypes had better above-mentioned cognitive functions than those who were GG homozygotes.

Cognitive functions vs interaction between *APOE* and *ESR1* polymorphisms

Memory, visual memory, processing and psychomotor speeds depended significantly on the interaction between *APOE* and *ESR1 Pvu*II polymorphisms (Table III and Figure 1). In women carrying the C allele of the *Pvu*II polymorphism (TC and CC) cognitive functions were the best in the presence of the ε2/ε3 genotype, lower if they possessed ε3/ε3 and the lowest if they possessed

Table III. Cognitive functions vs. *ESR1 Pvu*II polymorphism and vs. interaction with *APOE* polymorphism

Cognitive function	*Pvu*II						Comparisons of cognitive functions			
	TT		TC		CC		Vs. *Pvu*II		Vs. *Pvu*II * *APOE*	
	Mean	SD	Mean	SD	Mean	SD	*F*	*P*-value	*F*	*P*-value
NCI	81.2	18.2	84.0	17.1	83.8	17.2	0.657	0.519	0.740	0.565
Memory	89.2	18.2	89.5	15.6	84.3	17.1	2.368	0.096	2.657	0.033
Verbal memory	89.5	17.8	91.2	19.8	86.7	19.6	1.226	0.295	0.724	0.576
Visual memory	93.6	18.9	92.7	14.4	88.9	12.8	1.946	0.145	2.886	0.023
Processing speed	80.5	12.8	80.8	14.4	75.7	16.3	3.064	0.048	3.117	0.016
Executive functioning	72.2	27.5	80.7	25.1	81.2	24.4	3.084	0.047	0.512	0.727
Psychomotor speed	82.8	16.7	82.1	19.1	84.2	18.8	0.270	0.763	3.028	0.018
Reaction time	88.6	15.4	85.4	15.7	89.8	17.7	1.881	0.155	1.753	0.139
Complex attention	75.3	29.9	83.8	28.1	82.1	27.7	2.138	0.120	0.230	0.921
Cognitive flexibility	71.1	28.4	79.4	26.8	79.5	25.4	2.995	0.050	0.448	0.774

Table IV. Cognitive functions vs. *ESR1 Xba*I polymorphism and vs. interaction with *APOE* polymorphism

Cognitive function	*Xba*I						Comparisons of cognitive functions			
	AA		AG		GG		Vs. *Xba*I		Vs. *Xba*I * *APOE*	
	Mean	SD	Mean	SD	Mean	SD	*F*	*P*-value	*F*	*P*-value
NCI	84.7	16.7	83.4	18.0	79.3	17.0	1.501	0.225	2.494	0.050
Memory	90.9	17.8	88.8	15.6	79.8	16.3	7.111	0.001	1.598	0.175
Verbal memory	91.5	18.7	91.0	19.0	81.4	19.3	5.039	0.007	1.219	0.303
Visual memory	94.2	17.9	91.9	13.9	87.3	13.6	3.043	0.049	2.519	0.042
Processing speed	80.4	13.4	80.9	13.9	72.6	17.4	6.011	0.003	1.798	0.130
Executive functioning	76.8	24.7	80.3	27.5	76.3	23.0	0.653	0.521	1.035	0.390
Psychomotor speed	84.8	17.0	82.6	18.6	79.7	19.8	1.213	0.299	3.771	0.005
Reaction time	89.3	13.7	86.7	16.9	86.2	19.1	0.841	0.433	2.058	0.087
Complex attention	80.5	27.3	82.5	29.3	77.4	29.4	0.561	0.572	1.169	0.325
Cognitive flexibility	75.9	25.7	78.8	28.9	74.7	24.4	0.545	0.581	1.258	0.287

the ε4 allele. Different relations were observed in women with TT genotype of the *Pvu*II polymorphism – cognitive functions were not decreased in women with the ε4 allele.

Similarly, interaction between *APOE* and *ESR1 Xba*I polymorphisms had a significant impact on NCI, visual memory and psychomotor speed (Table IV and Figure 2). In women with AG and GG genotypes of the *Xba*I polymorphism cognitive functions were the best if women possessed *APOE* ε2/ε3 genotype, lower in the presence of ε3/ε3 genotype, and the lowest if they possessed the ε4 allele. Different relations were observed in women having AA genotype of the *Xba*I polymorphism for which cognitive functions were not decreased in women with the ε4 allele.

Cognitive functions vs interaction between *Pvu*II and *Xba*I polymorphisms of *ESR1* gene

NCI and six cognitive functions differed between genotypes of *Pvu*II and *Xba*I combined (Table V). Women who simultaneously carried CC *Pvu*II and GG *Xba*I genotypes had the lowest cognitive functions mentioned above, women with TC and AA or CC and AG demonstrated the best, while women with TT and AA or TC and AG had moderate outcomes.

Discussion

Old age is a risk factor for cognitive decline. In the aging population, there is a high prevalence of mild cognitive impairment (MCI), which increases

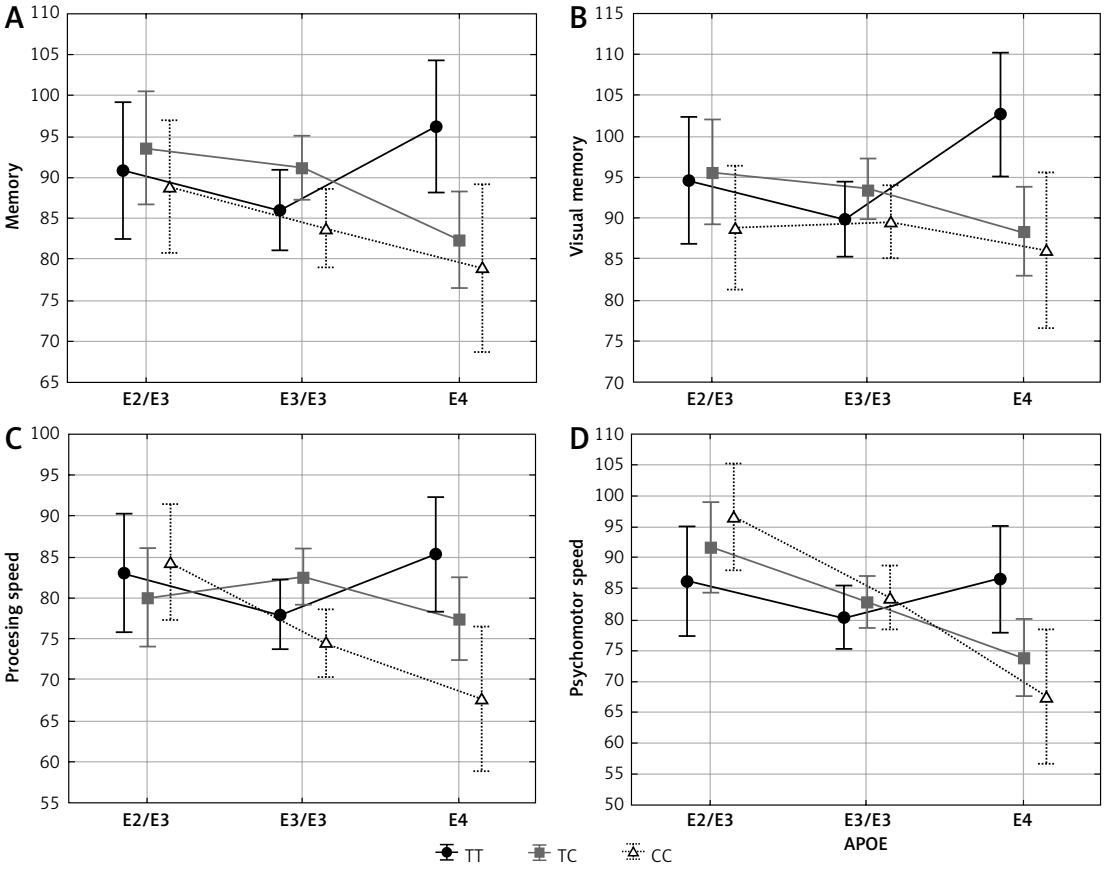

Figure 1. Significant effect of interaction between *APOE* and *ESR1 PvuII* polymorphisms on cognitive functions

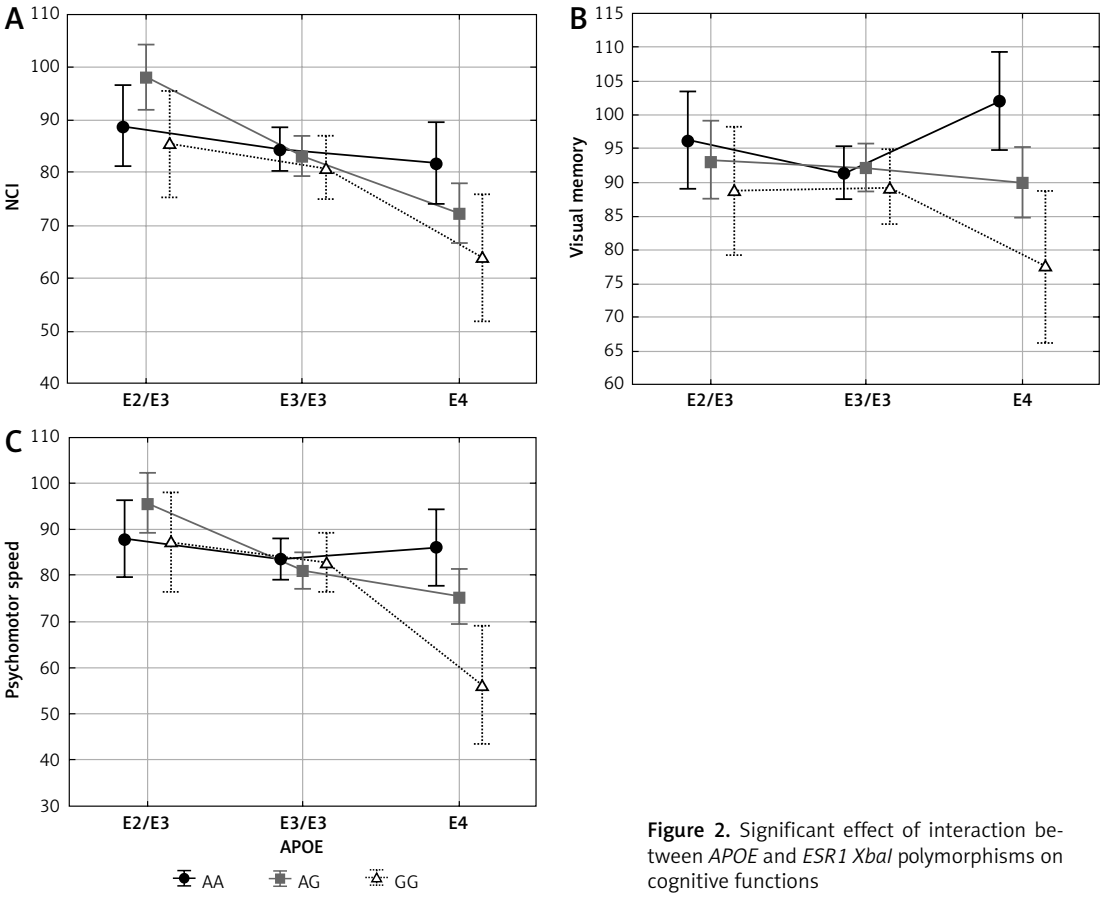

Figure 2. Significant effect of interaction between *APOE* and *ESR1 XbaI* polymorphisms on cognitive functions

Table V. Cognitive functions vs. interactions of *Pvu*II and *Xba*I of *ESR1* polymorphism

Cognitive function	TT and AA		TC and AG		CC and GG		TC and AA		CC and AG		Comparisons between groups	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	*F*	*P*-value
NCI	82.7	17.8	82.4	17.9	79.6	17.2	90.7	10.7	90.1	15.3	2.805	0.026
Memory	89.2	18.6	87.9	15.6	80.1	16.6	95.9	14.4	90.6	16.2	3.995	0.004
Verbal memory	89.5	18.3	89.7	19.8	81.6	19.6	97.6	19.2	94.4	17.2	3.403	0.010
Visual memory	93.7	19.5	91.9	14.9	87.6	13.8	95.9	11.8	90.8	11.2	1.446	0.219
Processing speed	80.2	12.7	80.7	14.3	72.9	17.8	81.0	15.4	79.9	13.0	2.500	0.043
Executive functioning	74.4	26.5	79.8	26.7	76.5	23.4	84.3	16.6	88.2	24.5	1.940	0.104
Psychomotor speed	82.7	17.3	80.0	19.4	79.6	20.2	91.2	14.7	91.0	14.2	3.640	0.007
Reaction time	88.5	14.5	84.0	16.3	87.4	18.5	91.4	11.3	93.4	16.2	2.622	0.035
Complex attention	77.2	29.3	82.3	29.9	77.5	29.8	90.3	17.6	88.9	23.2	1.692	0.152
Cognitive flexibility	73.1	27.7	78.3	28.7	74.8	24.9	84.1	16.1	86.6	24.9	1.810	0.127

Other combinations of PvuII and XbaI were not analyzed due to the very small sample sizes or even empty cells (N = 2 for TT and GG, N = 4 for TT and AG, N = 0 for CC and AA, N = 0 for TC and GG).

the risk of AD. It is forecast that by 2050 over 100 million people will develop such cognitive problems. MCI and dementia have consequences, especially for affected people but also for their caregivers, the health care delivery system and society in general. The problem especially applies to women after menopause when levels of estrogen, which have a neuroprotective effect, begin to drop significantly. In about 80% of women in the postmenopausal state neuronal degeneration may lead to difficulties in concentrating, overreacting or forgetfulness [21]. Menopause affects various activities of women's life [22]; among its effects, a decline in neurocognitive functions during the peri- and postmenopausal periods is observed [23].

The latest experimental data indicate some morphological changes in the hippocampus of postmenopausal female mice, i.e. mitochondrial damage, lipofuscin deposition and microtubule degradation [24]. In turn, in the study of Albert *et al.* [25], the effect of estrogen on the hippocampus and cognitive function was confirmed. The authors observed increased bilateral posterior hippocampal voxel-based gray-matter volume in women taking 2 mg of estrogen, while in women who received a placebo or 1 mg of estrogen no such effect was noted [25].

Age-related cognitive functions depend on various genetic risk factors, including *APOE* and *ESR1*. The first purpose of the present study was to analyze whether there are relations between both *APOE* and *ESR1* polymorphisms and several cognitive functions in a large group of postmenopausal women. We observed that women with ε4/ε4 show significantly lower executive functioning, psychomotor speed, reaction time, complex attention as well as cognitive flexibility compared to those with other *APOE* genotypes. The study of Schoemaker *et al.* [26] revealed significant positive correlations between familiarity performance and the volume of the perirhinal and entorhinal cortices as well as between recollection performance and hippocampal volume in carriers of *APOE* ε4. Levels of ApoE were found to be higher in women and related to lifespan and cognitive function [27]. In turn, in elderly women who were carriers of the *APOE* ε4 allele and exposed to traffic pollution, cognitive impairment in the visuospatial domain was demonstrated [28]. Similarly, the presence of some depressive symptoms has a significant effect on cognitive impairment, which is increased in *APOE* ε4 carriers [29]. It was also demonstrated that in post-menopausal women the correlation between CRP level and cognitive functions may be modified by apolipoprotein E genotypes [30]. In addition, an enhanced negative effect of testosterone on cognition was found in postmenopausal women with at least one *APOE* ε4 allele [31].

In the case of *ESR1* polymorphisms we observed that memory, verbal memory, visual memory and processing speed were at the lowest level in G allele homozygotes of the *Xba*I polymorphism. In contrast, women with CC genotype of the *Pvu*II polymorphism had lower processing speed and cognitive flexibility but higher executive functioning. Data regarding the *ESR1* polymorphisms are often contradictory. Some studies show such a relationship while others do not. In the study of Elcoroaristizabal *et al.* [32] an association between the *APOE* ε4 allele and amnesic mild cog-

nitive impairment was found while no relation to cognition was observed for *ESR1* polymorphisms in elderly women. In contrast, in the same study the polymorphisms within the *ESR2* gene were associated with lower cognitive performance [30]. Similar findings were described by Ryan *et al.* [33]. No relationship between *ESR1* polymorphisms and AD or vascular dementia was found in older Jewish women [34]. On the other hand, the study of Ma *et al.* [9] analyzing the same relationship between twenty *ESR1* polymorphisms and cognition in a group of Chinese older adults demonstrated that eight polymorphisms may be considered as markers for episodic memory decline at an earlier stage. In the study of Yaffe *et al.* [35] cognitive impairments were more likely to occur in women than men. The rs9340799 (*Xba*I) polymorphism in the *ESR1* gene was associated with cognitive decline in univariate analysis and after adjustment for age, educational level and score of 3MS (Modified Mini-Mental State) the correlation was slightly stronger. Such a relationship was not observed in the case of men [33].

The main goal of our study was to analyze the impact of the interaction between *APOE* and *ESR1* polymorphisms on cognitive functions in the analyzed women. In the case of interactions between *ESR1 Pvu*II polymorphism and *APOE*, significant differences in the domains of memory, visual memory, processing and psychomotor speeds were observed. The lowest cognitive functions characterized women with *Pvu*II TC and/or CC genotypes with simultaneous presence of at least one *APOE* ε4 allele. The best cognition was observed for women with TC and/or CC and ε2/ε3 genotypes. Otherwise, women with *Pvu*II TT seemed to be protected from cognitive decline even in the presence of the *APOE* ε4 allele, and their functions did not get lower with the *APOE* genotypes. Similar findings in regard to the interactions between *ESR1 Xba*I and *APOE* polymorphisms were demonstrated. Women with *Xba*I AG and/or GG genotypes and the *APOE* ε4 allele had the lowest cognition compared to women with *Xba*I AG and/or GG genotypes but having ε2 or ε3 alleles. In women with *Xba*I AA and *APOE* ε4, smaller deterioration in NCI as well as better visual memory and psychomotor speed was observed.

The study of Fernández-Martínez *et al.* [13] showed increased risk of mild dementia and AD in the simultaneous presence of the *APOE* ε4 allele and polymorphic variants of both *Xba*I and *Pvu*II polymorphisms within the *ESR1* gene. Opposite to our study, Fehsel *et al.* [11] found no relation of *ESR1* polymorphisms and cognition, but minor variants of *ESR2* polymorphisms had a significant impact on executive function in women carrying the *APOE* ε4 allele. In addition, the authors observed that air pollution increased the risk of cognitive decline in women. In male patients with AD, *ESR1* PP and XX genotypes increased the risk of the disease and the risk was especially high in those with at least one *APOE* ε4 allele (OR = 13.3) [36]. It was also demonstrated that wild-type genotypes (i.e. PP and XX) of *ESR1* of *Pvu*II and *Xba*I polymorphisms were associated with a faster cognitive decline in women with AD. Also, these genotypes decreased ApoE levels in male patients [36].

Cognitive functions in postmenopausal women may also correlate with biochemical, environmental or social factors. Previously, the impact of some of them, i.e. education, health behaviors, serum hormones and CRP protein concentrations, on cognitive functions was analyzed [23, 30, 31, 37]. The impact of cardiovascular risk factors on cognition was also considered [38, 39]. However, further studies are needed to understand the wide range of risk factors of cognition impairment as well as the interactions between them.

In conclusion, the AA and TT genotypes of estrogen receptor α polymorphisms protect postmenopausal women having the ε4 allele of the *APOE* polymorphism from low cognitive functions. NCI, memory, verbal memory, processing speed, psychomotor speed as well as reaction time were decreased the most in postmenopausal women who simultaneously carried the CC *Pvu*II and GG *Xba*I genotypes.

Conflict of interest

The authors declare no conflict of interest.

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