Neuroendocrinology Letters Volume 35 No. 8 2014
ISSN: 0172-780X; ISSN-L: 0172-780X; Electronic/Online ISSN: 2354-4716
Web of Knowledge / Web of Science: Neuroendocrinol Lett
Pub Med / Medline: Neuro Endocrinol Lett

Diabetes, hypertension and stroke – does Alzheimer protect you?

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Submitted: 2014-11-12 Accepted: 2014-12-12 Published online: 2015-01-18

Key words: **ApoE; hypertension; diabetes; stroke; association; polymorphism**

Neuroendocrinol Lett 2014; **35**(8):691–696 **PMID:** 25702297 NEL350814A03

Abstract

OBJECTIVE: The aim of our current research project is to further evaluate the role of risk factors in the pathogenesis of Alzheimer's disease; these include genetic variations, environmental factors and lifestyle issues.
METHODS: We have been conducting an association study on 373 patients with Alzheimer's disease and 286 unrelated control individuals. The occurrence and the age of onset of diabetes and cardiovascular diseases were evaluated in both groups. Apolipoprotein E genotype was analyzed in all subjects by PCR method.
RESULTS: We report that, in Czech population carrying ApoE4 allele increases risk of Alzheimer's disease 2.1-fold and genotype E4E4 increases the risk 8.4-fold. We have also identified a significant association between ApoE4 allele, Alzheimer's disease and hypertension. Hypertensive subjects with the ApoE4 allele have 1.5-fold greater risk of Alzheimer's disease. Thus, hypertension together with ApoE4 allele translates into 1.5-fold higher risk of AD. The most intriguing original finding in the present study is that Alzheimer's disease patients have significantly later onset of diabetes, hypertension and stroke in comparison with control subjects. This effect was not influenced by ApoE genotype. The diabetes appeared in AD patients on average more than 10 years later than in the control subjects ($p<0.0001$), hypertension was diagnosed 14 years later in AD patients ($p<0.00001$) and stroke occurred on average 6 years later ($p<0.005$), compared to the control group.
CONCLUSIONS: Overall, in addition to the above novel findings, our study expands the data base on risk factors that could be used in near future when testing for the genetic risk of Alzheimer's disease.

ORIGINAL ARTICLE

Abbreviations:

AD	- Alzheimer's disease
ANOVA	- analysis of variance
ApoE	- apolipoprotein E
ApoE4	- E4 allele of apolipoprotein E
APP	- amyloid precursor protein
bp	- base pair
CSS	- computer statistic software
CT	- computerized tomography
DMSO	- dimethyl sulfoxide
DNA	- deoxyribonucleic acid
ICD-10	- 10th revision of the International Statistical Classification of Diseases and Related Health Problems
MMSE	- minimental state examination
Ods	- odds ratios
OR	- odds ratio
PCR	- polymerase chain reaction

INTRODUCTION

As the average age and longevity of world population increase, the incidence and prevalence of Alzheimer's disease (AD) is on the rise. In the near future, this may lead to significant deterioration in the quality of human life at both individual and social level. Greater knowledge of AD pathogenesis and related risk factors would offer hope for better prevention of Alzheimer's disease and/or mitigation of some of the associated adverse effects leading to potentially very significant beneficial outcomes (Šerý *et al.* 2014).

Risk factors for the pathogenesis of Alzheimer's disease (AD) are multiple and wide-ranging, including cerebrovascular and cardiovascular diseases (particularly hypertension), smoking, high or moderate alcohol intake, obesity, hypercholesterolemia, diabetes mellitus, education and socioeconomic status, and both physical and mental activity (for review see Povova *et al.* 2013).

There are several specific examples of how the above factors can alter the risk of AD. The presence of type II diabetes has been reported as associated with an approximately twofold increase in the risk of AD (Mayeux & Stern 2012). The relationship of hypertension to AD has been assessed from two perspectives. The first aspect involved the study of the relationship between AD and hypertension in middle age, the second aspect focused on the hypertension in later life. The onset of hypertension during the middle age appears to increase the risk of AD pathogenesis in persons that do not have their blood pressure adequately treated and controlled (Feldstein 2012). The relationship between the late age onset hypertension and AD is more complex (Mayeux & Stern 2012); abnormally low blood pressure is also associated with dementia and it may be related to worsening of the brain hypoperfusion (Feldstein 2012). Recent studies have reported a close link between sporadic AD and ischemic brain episodes (Pluta *et al.* 2012). There are several explanations of vascular pathology in AD including iron-induced fibrin formation (Lipinski & Pretorius 2013; Lipinski & Pretorius 2014).

The apolipoprotein E genotype with known links to dynamics of cholesterol transport has been implicated as a vascular risk factor in influencing AD (Šerý *et al.* 2013). Cerebral hypoperfusion may initiate and/or accelerate the neurodegeneration cascade causing amyloid deposition, synaptic and neural dysfunction and lead to cognitive impairment (Pluta *et al.* 2013). Aβ deposition into the capillary wall is strongly associated with the ApoE4 allele as a risk factor (Attems *et al.* 2010).

The primary aim of the present study has been to investigate the effect of the ApoE genotype (rs 429358 and rs7412) on the risk of Alzheimer's disease with a particular focus on a possible correlation between AD and the occurrence of hypertension, stroke, type II diabetes and selected lifestyle risk factors.

MATERIALS & METHODS

Subjects

We examined a total of 373 patients with Alzheimer's disease and 286 unrelated control individuals originating from the Czech Republic. The group of patients with AD (age 79.5±8.1) was diagnosed according to the criteria of International Classification of Diseases 10th Revision (ICD-10); additionally 211 patients underwent CT scans to verify the diagnosis. Patients were hospitalized with diagnosis of AD in Psychiatric hospitals Kroměříž, Šternberk, Jihlava, Moravský Beroun and Opava. Control subjects (age 70.8±9.6) were enrolled in various other departments (eye clinics, traumatology clinic) in hospitals Kroměříž, Olomouc and in the Faculty Hospital of Masaryk University in Brno-Bohunice. Some control subjects were life partners (spouses) of AD subjects. In the control group the presence of dementia was excluded using the minimental state examination (MMSE) and interviews with psychiatrists. Informed consent was obtained from all participants or their legal representatives and formal approval for the study was granted by the Ethics Committee of the Faculty of Medicine, University of Ostrava.

The questionnaires for both groups – patients and controls – contained questions about vascular risk factors such as smoking, alcohol consumption, weight and height, the age of onset of diabetes, stroke and hypertension and its therapy, selected nutritional factors, presence of other cardiovascular and cerebrovascular diseases, and a history of head injury. Answers of AD patients were based on the medical records collected by physicians. The questions related to psychosocial factors such as education and socioeconomic level, social network and the extent of social engagement of each individual, as well as those related to physical and mental activities were also included in the questionnaire.

Genotyping

DNA from buccal swabs was isolated according to the modified protocol of Prepito NA Body Fluid Kit on

automated system Prepito (chemagen – Perkin Elmer, USA). The ApoE gene sequence with rs 429358 and rs7412 polymorphic sites was amplified using the polymerase chain reaction (PCR). Each PCR mix contained 1 µl of extracted DNA (50 ng/µl), DNA polymerase KAPA2G Robust HotStart (Kapa Biosystems, Japan), 1% 7-deaza-dGTP (Roche Diagnostics, USA), 5% DMSO and PCR water in final volume of 20 µl. The labelled oligonucleotide primers Apo1 FAM – 5´CGG ACA TGG AGG ACG TG 3´ and Apo2 TAMRA – 5´CCC CGG CCT GGT ACA CT 3´ were used. Special polymerase KAPA2G Robust HotStart, DMSO and 7-Deaza-dGTP were used for PCR mixture because of high content of G+C bases in nucleotide sequence of ApoE gene. The PCR conditions were: initial denaturation at 95 °C for 10 min, followed by 35 cycles of denaturation 95 °C for 1 min, annealing at 58°C (with 20% ramp) for 30 s and elongation at 72 °C for 60 s. A final extension at 72 °C for 7 min was included.

The PCR products were digested with Hha 1 restriction enzyme to recognize rs 429358 and rs7412 polymorphisms. The mixture was incubated at 37 °C for 30 min and 95 °C for 5 min. Restricted DNA fragments were denatured by formamide. Finally, fragments were separated by capillary gel electrophoresis on an automated Genetic Analyzer ABI 3130 (Applied Biosystems, USA).

Statistics

The CSS Statistica software (StatSoft, USA) was used for statistical evaluation of the results. The chi-square test and Fisher's exact test were used for the comparison of genotype and allele frequencies in the studied groups. Odds ratios (Ods) and 95% confidence intervals (95% CI) as estimates of the relative risk for the AD associated with different genotypes were calculated with logistic regression. One-way analysis of variance (ANOVA) was used to determine whether there are any significant differences between the means of two or more independent groups.

RESULTS

The comparison between AD patients and control subjects in scores of the minimental state examination (MMSE) is shown in Figure 1 ($p<0.00001$).

ApoE genotype was successfully analyzed in all 659 subjects and separate association analyses between ApoE genotype and Alzheimer's disease, diabetes, hypertension and stroke are displayed in Table 1. The frequency of ApoE4 allele is significantly higher in AD patients in comparison with control subjects ($p=0.00001$). The presence of ApoE4 allele in the genotype significantly ($p<1 \times 10^{-9}$, two tailed Fisher Exact Probability Test) increases the risk of AD 2.1-fold (Odds Ratio = 2.9324; 95% CI of OR = 2.0745 till 4.1449, Risk Ratio = 2.0569; 95% CI of OR = 1.6101 till 2.6276). The ApoE gene genotype E4E4 significantly ($p<0.02$) increases risk of AD in carriers 8.4-fold (Odds Ratio = 8.6602; 95% CI of OR = 1.1115 till 67.4758, Risk Ratio = 8.4343; 95% CI of OR = 1.0952 till 64.9526). The ApoE gene genotype E3E4 increases risk of AD

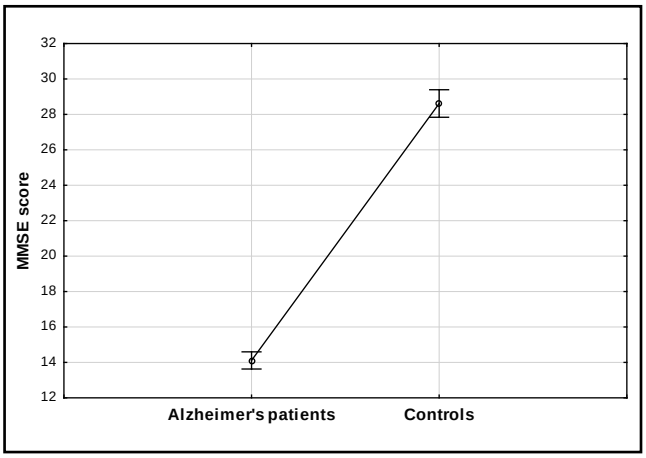

Fig. 1. The comparison of MMSE score between Alzheimer's patients and controls. F=969.06; $p<0.00001$.

Tab. 1. The relationship between cases and controls for Alzheimer's disease, diabetes, hypertension and stroke in the ApoE genotype and alleles.

		ApoE genotype						Frequency of E2, E3, E4 alleles (%)			F-test	χ2
		E2/E2	E2/E3	E2/E4	E3/E3	E3/E4	E4/E4	E2	E3	E4		
AD	case	1	26	11	177	147	11	5.2	70.6	24.1	<0.00001	0.26
	control	2	50	6	171	56	1	10.4	78.3	11.2		
diabetes	case	0	15	7	90	58	3	6.3	73.1	20.6	0.76	0.068
	control	3	41	10	217	132	9	6.9	73.6	19.4		
hypertension	case	2	54	13	239	141	6	7.8	73.9	18.2	0.68	0.066
	control	1	22	4	97	61	6	7.3	72.5	20.2		
stroke	case	0	9	4	52	25	4	6.9	73.4	19.7	0.43	0.099
	control	3	66	13	284	177	8	7.7	73.6	18.7		

two-fold (Odds Ratio = 2.6715; 95% CI of OR = 1.8668 till 3.8229, Risk Ratio = 2.0127; 95% CI of OR = 1.5419 till 2.6273, $p<1 \times 10^{-7}$). In contrast, the risk of AD is significantly decreased (0.4-fold; $p<0.0001$) in carriers of E2E3 genotype (Odds Ratio = 0.3537; 95% CI of OR = 0.2141 till 0.5842, Risk Ratio = 0.3987; 95% CI of OR = 0.2547 till 0.6242).

ApoE genotype has not been found to be associated with diabetes, hypertension and stroke in mixed groups of AD subjects and controls (Table 1). Neither have we found any significant relationship when we grouped the subjects into AD patients and controls and studied allelic frequency differences between ApoE and diabetes, hypertension and stroke separately in each group (Table 2). The last section of Table 2 represents the frequency of ApoE genotype in diabetes, hypertensive and stroke patients in the group of AD patients and the same relationships in the group of AD controls. We found a significant association between ApoE genotype and hypertension, when we compared hypertensive patients in AD group and AD controls ($p<0.0001$), see Table 2. In the group of all hypertensive subjects the presence of ApoE4 allele statistically significantly ($p=0.000001$) increases risk (1.5-fold) of Alzheimer's disease (Odds Ratio = 2.7912; 95% CI of OR = 1.8459 till 4.2204, Risk Ratio = 1.5038; 95% CI of OR = 1.2895 till 1.7536). The presence of ApoE4 allele together with hypertension increases the risk of AD 1.5-fold.

The risk of AD in relatives of AD patients with presence of ApoE4 allele is 2.82× higher (Odds Ratio = 3.0658; 95% CI of OR = 1.3968 till 6.7291, Risk Ratio = 2.8256; 95% CI of OR = 1.3568 till 5.8842). Tobacco smoking had a marginal but statistically significant ($p=0.001$) effect increasing the risk of AD in our group of subjects 1.13-fold (Odds Ratio = 1.8935; 95% CI of OR = 1.2881 till 2.7834, Risk Ratio = 1.1295; 95% CI of OR = 1.0459 till 1.2198).

We found that the onset of diabetes, hypertension and stroke in patients with AD took place later than in the control group. This effect was not influenced by ApoE genotype. The diabetes appeared in AD patients on average more than 10 years later than in the control subjects ($p<0.0001$), hypertension was diagnosed 14 years later in AD patients ($p<0.00001$) and stroke occurred on average 6 years later ($p<0.005$), compared to the control group (Figures 2–4).

Tab. 2. The comparison of occurence of diabetes, hypertension and stroke between ApoE genotypes in the group of Alzheimer's patients and controls.

		ApoE genotype						
		E2/E2	E2/E3	E2/E4	E3/E3	E3/E4	E4/E4	χ2
Alzheimer's disease group								
diabetes	yes	0	8	5	49	44	3	0.075
	no	1	18	6	128	103	8	
hypertension	yes	1	17	8	123	102	6	0.098
	no	0	9	3	53	44	6	
stroke	yes	0	4	2	31	18	3	0.091
	no	1	21	9	145	128	8	
Group of controls								
diabetes	yes	0	7	2	41	14	0	0.101
	no	2	23	4	89	29	1	
hypertension	yes	1	37	5	116	39	1	0.074
	no	1	13	1	44	17	0	
stroke	yes	0	5	2	21	7	1	0.183
	no	2	45	4	139	49	0	
Alzheimer's disease patients (AD) vs. controls (cont.)								
diabetes	cont.	0	7	2	41	14	0	0.049
	AD	0	8	5	49	44	3	
hypertension	cont.	1	37	5	116	39	1	$p<0.0001$
	AD	1	17	8	123	102	6	
stroke	cont.	0	5	2	21	7	1	0.578
	AD	0	4	2	31	18	3	

DISCUSSION

Many studies have reported diabetes, hypertension and stroke as risk factors in the pathogenesis of Alzheimer's disease. We decided to analyze the relationship between these cardiovascular diseases and apolipoprotein E genotypes in a group of 659 AD patients and controls of Czech origin. ApoE genotype frequencies in our study (E2E2 0,005, E2E3 0.115, E2E4 0.026, E3E3 0.528, E3E4 0.308, E4E4 0.018) were similar to the frequencies recently reported for Czech population by Hubáček *et al.* (2013) – E2E2 0.007, E2E3 0.117, E2E4 0.02, E3E3 0.66, E3E4 0.187, E4E4 0.009. Hubáček *et al.* (2013) studied the association between ApoE genotype and left-handedness on general Czech population and did not specifically focus on any pathological condition including AD. Somewhat higher count of genotypes containing ApoE4 allele present in our data may be caused by the particular design of our study – we had a higher number of AD patients in our group of subjects.

The data in the present study are in accordance with previously extensively described relationship between ApoE genotype and Alzheimer's disease (reviews: Šerý *et al.* 2013; Armstrong 2013). We found that ApoE4 allele occurs at significantly higher frequency in AD patients in comparison with control subjects (p=0.004). In quantitative terms the results of our study translate into ApoE4 allele presence in genotype increasing the risk of AD 2.1-fold ($p<1 \times 10^{-9}$). Carrying of the genotype E4E4 increases the risk of AD 8.4-fold (p<0.02). AD patients in our study carrying ApoE4 allele were found to have 2.82-times greater number of relatives with AD in comparison with control subjects. Our data are comparable to those obtained using other population groups (for recent review see Raichlen & Alexander 2014).

Tobacco smoking also significantly (p=0.001) increased the risk of AD (the effect was not very strong, though; 1.13-fold difference) in our group of subjects; this is in agreement with the findings of recent studies (Povová *et al.* 2012).

Dembińska-Kieć *et al.* (1998) were the first to suggest that the ApoE phenotypes and insulin output may contribute to an early detection of individuals at high risk of hypertension development. Their study suggested that elevated levels of blood pressure in middle age can increase the risk of late age-onset dementia in men never treated with anti-hypertensive medication. Launer *et al.* (2000) found the relationship between elevated levels of blood pressure in middle age and an increased risk of dementia at later age in men never treated with anti-hypertensive medication. Yilmaz *et al.* (2001) reported a trend for ApoE4 allele to be associated with a higher prevalence of target organ damage in patients with mild to moderate hypertension. Li *et al.* (2003) reported similar findings in Chinese population. In hypertensive patients with apolipoprotein E3E4 and E4E4 genotypes, systolic blood pressure was significantly higher than in those with apolipoprotein E2E3 or E3E3 genotypes.

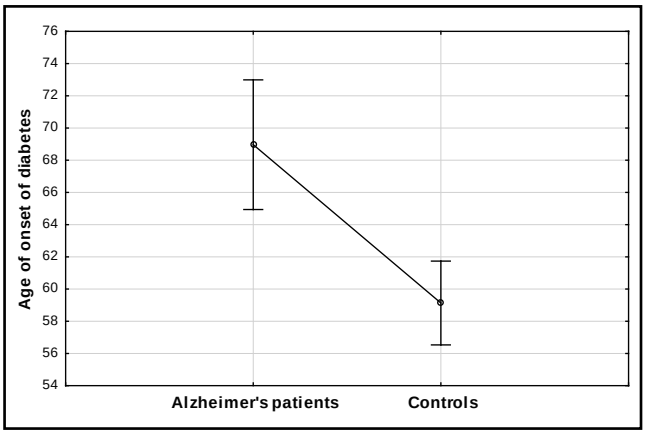

Fig. 2. The comparison of age-onset of diabetes between Alzheimer's patients and controls. F=16.590, p=0.0001.

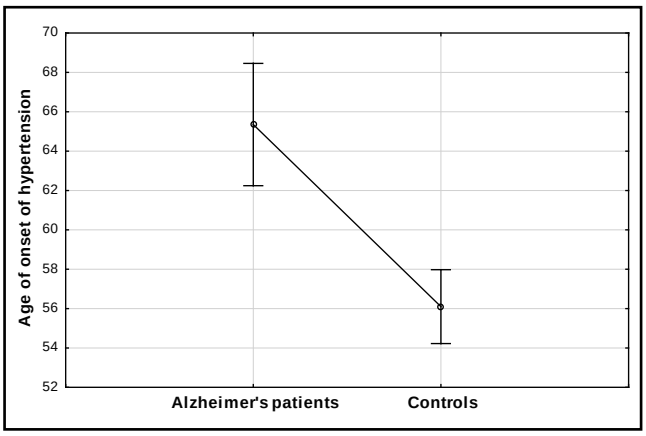

Fig. 3. The comparison of age-onset of hypertension between Alzheimer's patients and controls. F = 25.264, p<0.00001.

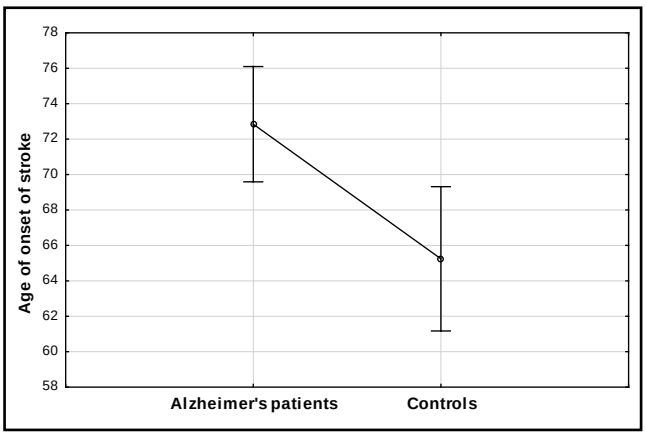

Fig. 4. The comparison of age-onset of stroke between Alzheimer's patients and controls. F = 8.3891, p=0.00471.

In our study the presence of ApoE4 allele combined with hypertension increases the risk of AD 1.5-fold. Our findings may be explained by those of Kesler *et al.* (2010). Kesler *et al.* (2010) reported that in ApoE4 allele carriers, hypertension was associated with higher

tau and ptau-181 levels in cerebrospinal fluid. In their study, the hypertension was not associated with Abeta42 levels, and ApoE genotype did not modify this relationship. Kester *et al.* (2010) suggested that the hypertension is directly related to Tau pathology in ApoE4 homozygous carriers. ApoE4 allele seems to mediate hyperphosphorylation of Tau protein (Rahman *et al.* 2005). Hypertension itself in ApoE4 carriers seems to accelerate AD pathophysiology via higher Tau protein levels.

What seems to be a very curious and unusual finding in the present study is that AD patients have significantly delayed onset of diabetes, hypertension and stroke in comparison with the control subjects. This effect appears to be unrelated to the ApoE genotype. It is based on histories of patients collected from their hospital and clinical records. We concede that the records might contain occasional errors, but the difference that we found is so large and the associated statistical significance so high that we do not believe that the result could have been produced merely by inaccuracies in the patients' records. Are cardiovascular diseases really risk factors of AD? Yes, indeed; our study does not cast serious doubt on that relationship *per se*. It does appear, however, that patients with AD may be "protected" against cardiovascular diseases and diabetes for a period of time but, as soon as the cardiovascular disease or diabetes appear, they are quickly followed by the onset of AD symptoms. A possible explanation of one of the present findings is offered by recent results of Wang *et al.* (2014); insulin could inhibit Abeta production through modulation of APP processing by increasing APP cleavage at the alpha-secretase site and decreasing the cleavage at the beta-secretase site. Disturbances in the insulin levels associated with the onset of diabetes could then accelerate the development of AD pathology and bring forward the time when it first manifests itself as dementia.

Stated in more general terms, persons at high risk of AD may experience a degree of apparent protection (mediated by as yet unidentified factors) from cardiovascular disease and diabetes during their middle or late-middle age but, when those conditions develop they are rapidly followed by onset/deterioration in AD. We have at present no mechanistic explanation of this relationship at a molecular and cellular level but, based on available patient data, it does not appear to have been caused by any obvious trivial factors.

ACKNOWLEDGMENTS

This work has been supported by Internal grant agency of the Ministry of Health of the Czech Republic (IGA MZCR) No. NT/11152-6.

REFERENCES

1 Armstrong RA (2013). What causes Alzheimer's disease? Folia Neuropathol. **51**: 169–188.
2 Attems J, Yamaguchi H, Saido TC, Thal DR (2010). Capillary CAA and perivascular Aβ-deposition: two distinct features of Alzheimer's disease pathology. J Neurol Sci. **299**: 155–162.
3 Dembińska-Kieć A, Kawecka-Jaszcz K, Kwaśniak M, Guevara I, Pankiewicz J, Malczewska-Malec M, et al (1998). Apo E isoforms, insulin output and plasma lipid levels in essential hypertension. Eur J Clin Invest. **28**: 95–99.
4 Feldstein CA (2012). Association between chronic blood pressure changes and development of Alzheimer's disease. J Alzheimers Dis. **32**: 753–763.
5 Hubáček JA, Piper BJ, Pikhart H, Peasey A, Kubinova R, Bobak M (2013). Lack of an association between left-handedness and APOE polymorphism in a large sample of adults: results of the Czech HAPIEE study. Laterality. **18**: 513–519.
6 Kester MI, van der Flier WM, Mandic G, Blankenstein MA, Scheltens P, Muller M (2010). Joint effect of hypertension and APOE genotype on CSF biomarkers for Alzheimer's disease. J Alzheimers Dis. **20**: 1083–1090.
7 Launer LJ, Ross GW, Petrovitch H, Masaki K, Foley D, White LR, et al (2000). Midlife blood pressure and dementia: the Honolulu-Asia aging study. Neurobiol Aging. **21**: 49–55.
8 Li X, Du Y, Du Y, Huang X (2003). Association of apolipoprotein E gene polymorphism with essential hypertension and its complications. Clin Exp Med. **2**: 175–179.
9 Lipinski B, Pretorius E (2013). The role of iron-induced fibrin in the pathogenesis of Alzheimer's disease and the protective role of magnesium. Front Hum Neurosci. **7**: 735.
10 Lipinski B, Pretorius E (2014). Iron-induced fibrin formation may explain vascular pathology in Alzheimer's disease. Folia Neuropathol. **52**: 205.
11 Mayeux R, Stern Y (2012). Epidemiology of Alzheimer disease. Cold Spring Harbor Perspectives in Medicine. **2**.
12 Pluta R, Furmaga-Jabłońska W, Maciejewski R, Ułamek-Kozioł M, Jabłoński M (2013). Brain ischemia activates β- and γ-secretase cleavage of amyloid precursor protein: significance in sporadic Alzheimer's disease. Mol Neurobiol. **47**: 425–434.
13 Pluta R, Kocki J, Maciejewski R, Ułamek-Kozioł M, Jabłoński M, Bogucka-Kocka A, et al (2012). Ischemia signalling to Alzheimer-related genes. Folia Neuropathol. **50**: 322–329.
14 Povová J, Ambroz P, Bar M, Pavuková V, Šerý O, Tomášková H (2012). Epidemiological of and risk factors for Alzheimer's disease: a review. Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. **156**: 108–114.
15 Rahman A, Akterin S, Flores-Morales A, Crisby M, Kivipelto M, Schultzberg M, et al (2005). High cholesterol diet induces tau hyperphosphorylation in apolipoprotein E deficient mice. FEBS Lett. **579**: 6411–6416.
16 Raichlen DA, Alexander GE (2014). Exercise, APOE genotype, and the evolution of the human lifespan. Trends Neurosci. **37**: 247–255.
17 Šerý O, Povová J, Míšek I, Pešák L, Janout V (2013). Molecular mechanisms of neuropathological changes in Alzheimer's disease: a review. Folia Neuropathol. **51**: 1–9.
18 Šerý O, Povová J, Balcar VJ (2014). Perspectives in genetic prediction of Alzheimer's disease. Neuro Endocrinol Lett. **35**: 359–366.
19 Wang X, Yu S, Gao SJ, Hu JP, Wang Y, Liu HX (2014). Insulin inhibits Abeta production through modulation of APP processing in a cellular model of Alzheimer's disease. Neuro Endocrinol Lett. **35**: 224–229.
20 Yilmaz H, Isbir T, Ağaçhan B, Aydin M (2001). Is epsilon4 allele of apolipoprotein E associated with more severe end-organ damage in essential hypertension? Cell Biochem Funct. **19**: 191–195.