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Evaluation of *APOE* Genotype and Vascular Risk Factors As Prognostic and Risk Factors for Alzheimer's Disease and Their Influence On Age of Symptoms Onset

Gabriela Novotni[1*], Milena Jakimovska[2], Dijana Plaseska-Karanfilska[2], Nikolina Tanovska[1], Igor Kuzmanovski[1], Vasko Aleksovski[1], Katerina Karanfilovik[1], Natalija Baneva-Dolnenec[1], Marijana Stankovic[3], Milos Milutinovik[3], Svetlana Iloski[3], Rosalinda Isjanovska[4], Biljana Blazevska-Stoilkovska[5], Aleksej Duma[6], Antoni Novotni[3]

[1]University Clinic for Neurology, Clinical Centre Mother Theresa, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia; [2]Macedonian Academy for Science and Arts, Genetic Laboratory Prof. Dr Georgi Efremov, Skopje, Republic of Macedonia; [3]University Clinic for Psychiatry, Clinical Centre Mother Theresa, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia; [4]Institute for Epidemiology, Biostatistics and Medical Informatics, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia; [5]Faculty of Philosophy, Department of Psychology, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia; [6]Institute of Forensic Medicine, Criminology and Medical Deontology, Faculty of Medicine, Ss Cyril and Methodius University of Skopje, Skopje, Republic of Macedonia

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***Correspondence:** Gabriela Novotni. University Clinic for Neurology, Clinical Centre Mother Theresa, Faculty of Medicine, Ss Cyril and the Methodius University of Skopje, Skopje, Republic of Macedonia. E-mail: gabinovotni@gmail.com

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Abstract

BACKGROUND: Alzheimer's disease (AD), the most common cause of dementia, is evolving to become a threatening epidemy of the 21st century. Only 21% of the predicted number of AD patients in Macedonia have been diagnosed and treated, which means that almost 80% are underdiagnosed or misdiagnosed. Apolipoprotein E gene (*APOE*) is recognised as the strongest genetic risk factor for sporadic AD. Whether and when Alzheimer's disease develops, depends on the very complex interaction between genetic and modifiable risk factors. It has been known that vascular factors like hypertension, diabetes mellitus, hypercholesterolemia and obesity increase the risk of developing both AD, vascular dementia and mixed AD and vascular pathology

AIM: This study aims to evaluate the influence of *APOEε4* allele presence and modifiable vascular risk factors (hypertension, diabetes mellitus and dyslipidemia) as prognostic and risk factors for AD and their influence on the age of onset of AD symptoms among 144 AD patients from Macedonia.

MATERIAL AND METHODS: Study group of a total of 144 patients diagnosed with AD was evaluated. APOE genotyping was performed using *APOE* haplotype-specific sequence specific-primer (SSP)-PCR (Polymerase Chain Reaction) methodology. The non-standardized questionnaire was used to obtain information about demographics, lifestyle and modifiable risk factors that could influence disease onset and phenotype.

RESULTS: Statistically significant association was found between the presences of *APOEε4* allele in AD group versus controls. The presence of *APOEε4* allele increases the risk of developing AD in a 3-fold manner. The average age of disease onset in the ε4 carrier group was 67.2 ± 8.3 and in the ε4 non-carrier group 69.7 ± 9.4. This confirms that the presence of *APOEε4* allele shifts towards earlier disease onset, though the difference is not statistically significant. Out of the vascular risk factors, only hypertension was significantly associated with earlier AD onset. Out of total 144 patients, in 22.9% the first symptom onset was before the age of 65, that can be considered as early onset Alzheimer's Disease (EOAD), which is much higher than 5% for EOAD as most of the studies report.

CONCLUSIONS: The average age of disease onset of 68.4 years could be considered earlier than the average age of AD onset worldwide. Out of all the vascular risk factors analysed in this study, only hypertension and dyslipidemia were found to significantly increase the risk for developing AD and only the presence of hypertension influences the age of onset, shifting towards earlier disease onset. Public awareness campaigns should be organised to influence general population knowledge about Alzheimer's disease, early recognition and the influence of modifiable vascular risk factors.

Introduction

Alzheimer's disease (AD), the most common cause of dementia, is evolving to become a threatening epidemy of the 21st century, even though not an infectious disorder. In 2015, 47 million people had dementia worldwide, with predictions that this number would at least triple by the year of 2050, estimating that there will be around 131 million living with dementia, mostly AD [1]. A disease that was in details described by Alois Alzheimer in 1906 and was

considered to be a rare disease at that time is about to become a global medical and social problem, mostly affecting people living in low- and middle-income countries. Even though the exact aetiology and pathogenesis of Alzheimer's disease is still an unrevealed challenge for the neuroscience, it is well known that old age is a major risk factor for AD. With better medical treatment for cardiovascular, cancer and infectious disorders and a growing number of ageing populations worldwide, the rapid expansion of Alzheimer's disease shortly is expected.

The standardized age prevalence for dementia worldwide is 5-7% for aged 60 years and more [1], that assumes that there are around 20,000 people living with AD in Macedonia (according to State Statistical Office, R. Macedonia 2.4.15.10/821, there are around 400,000 people aged 60 and more living in Macedonia). According to the data from Health Insurance Fund of Macedonia for donepezil (acetylcholinesterase inhibitor, registered for treatment of AD) prescriptions in 2017, only 4,200 patients were treated. This assumes that only 21% of the predicted number of AD patients in Macedonia have been diagnosed and treated, which means that almost 80% are underdiagnosed or misdiagnosed.

Two forms of Alzheimer's Disease are recognised according to the age of onset. Early-onset Alzheimer's disease (EOAD) that begins before the age of 65 and presents only 5% of overall AD patients. EOAD patients usually have positive family history for the disease, and in half of the EOAD, that is in only 1- 2% of the total AD patients, the disease develops due to a monogenic deterministic mutation in one of the three known genes amyloid precursor protein (*APP*), prseniline1 (*PSEN1*) or presenilin2 (*PSEN2*) gene.

According to another study, the percentage of autosomal dominant inherited forms of EOAD is even smaller, only around 7% of all early-onset cases [2]. The other 95% of AD patients, have disease onset at, or after the age of 65, late-onset Alzheimer's disease (LOAD), and present the majority of AD patients [1]. LOAD is sporadic, occurring in individuals with genetic susceptibility in complex interaction with environmental and lifestyle modifiable risk factors.

Apolipoprotein E gene (*APOE*) is recognised as the strongest genetic risk factor for LOAD. It has three allelic variants ε2, ε3 and ε4. It is the presence of *APOEε4* allele that increases the risk for LOAD. According to several studies, the presence of *APOEε4* allele in LOAD patients is 50-60% compared to 20-25% in healthy older adults respectively. The presence of ε4 allele increases the risk of developing AD in a dose-dependent manner. *APOEε4/ε4* homozygosity increases the risk for developing AD 14-fold, and APOE ε3/ε4 heterozygosity increases the lifetime risk for AD, 4-fold in comparison to ε3 homozygosity [3], [4], [5]. The presence of *APOEε4* allele shifts the age of disease onset approximately 5 to10 years earlier in heterozygosity, and up to 10-20 years earlier in homozygosity [2]. *APOEε4* allele frequency is highly variable in different population and ethnic groups [6]. The worldwide frequency of ε2, ε3 and ε4 alleles is 8.4%, 77.9% and 13.7%, respectively, but in AD patients the ε4 frequency increases up to 40% [7]. When discussing the APOE gene as a risk factor for AD, it must be stressed that it only influences the individual's genetic susceptibility, but it is not deterministic as the previously mentioned three gens. That means, that even if homozygosity for ε4 is present, it only increases lifetime risk, but does not mean that AD would certainly develop.

Whether and when Alzheimer's disease develops depends on the very complex interaction between genetic and the modifiable risk factors. It has been known that vascular risk factors like hypertension, diabetes mellitus, hypercholesterolemia and obesity increase the risk of developing both AD, vascular dementia and mixed AD and vascular pathology [6].

This study aims to evaluate the influence of APOEε4 allele presence and modifiable vascular risk factors (hypertension, diabetes mellitus and dyslipidemia) as prognostic and risk factors for AD and their influence on age of symptoms onset among 144 AD patients from Macedonia.

Material and Methods

The study group includes 144 subjects that were diagnosed in the dementia outpatient clinic at the University Clinic of Neurology-Skopje and dementia centre at the University Clinic of Psychiatry-Skopje within the period from 2016 to 2018. All subjects fulfilled criteria for probable Alzheimer's dementia according to standard diagnostic criteria [8]. A standard procedure of blood sample collection was performed for DNA isolation. APOE genotyping was performed in the genetic laboratory "Prof. Dr Georgi Efremov", Macedonian Academy of Arts and Sciences. *APOE* haplotype-specific sequence specific-primer (SSP)-PCR (Polymerase Chain Reaction) methodology was used to determine the three main APOE isoforms. The non-standardized questionnaire was used to obtain information about demographics, lifestyle and modifiable risk factors that could influence disease onset and phenotype. We used an age-matched control group to evaluate ε4 allele frequency.

Written informed consent was obtained from all subjects included in the study group and from the control group.

Statistical analysis in STATISTICA 7.1, SPSS 20.0 were done, using chi-square test, t-test and

univariate and multiple logistic regression analyses.

Results

A study group of a total of 144 patients diagnosed with AD was evaluated. *APOEε4* allele carriers were found to be 72 out of 144 AD patients, that is 50.0%, compared to the control group where *APOEε4* allele carriers were 21 out of 90 individuals, which makes 23.3%. Statistically, a significant association was found between the presence of *APOEε4* allele and AD, $p < 0.05$ (Pearson Chi-square = 16.8103, p = 0.000041). 9% of all AD patients in our study group were *APOEε4/ε4* homozygotes, versus only 1 subject (1.1%) in the control group. Calculated *APOEε4* allele frequency was 30.14% in the AD patients' group, compared to 12.22% in the control group, which is statistically significant ($p < 0.05$).

APOE genotype/AD patients	Count	%
e3-e3	67	46.5
e3-e4	59	41.0
e2-e3	4	2.8
e4-e4	13	9.0
missing	1	0.7
total	144	100.0
APOE genotype/controls		
e3-e3	60	66.7
e3-e4	19	21.1
e2-e3	9	10.0
e2-e4	1	1.1
e4-e4	1	1.1
total	90	100.0

Figure 1: APOE genotype distribution among AD patients and controls

According to the analysis in our study group, the presence of *APOEε4* allele increases the risk of developing AD in a 3-fold manner [OR-3.3320 (1.8502-6.0005)]. *APOEε4* undoubtedly has influenced the genetic susceptibility in sporadic AD patients and has influenced the disease manifestation.

The average age of onset of Alzheimer's disease in our study group, which makes the first AD research till now in this geographic region and among this ethnic population is 68.4 years. This can be considered lower in comparison to Nussbaum reports that the average age of dementia onset is around 80 years [2].

One of the explanations could be that younger patients are referred to the dementia outpatient clinic at the University Clinic of Neurology and dementia centre at the University Clinic of Psychiatry, and older patients with Alzheimer's disease are under or miss diagnosed. Unfortunately, the public awareness about Alzheimer's disease in Macedonia is still low, and forgetfulness is still considered as a part of normal ageing.

Even though this might seem like a logical explanation, it is unlikely that only younger AD patients were referred to tertiary level, as this research lasted for 2 years, and in the meantime, a lot of educational workshops for family doctors were organised influencing the professional awareness about AD. If we conclude that sporadic AD in Macedonia has a somewhat earlier onset than in other population, it is important to identify the causes. As the presence of *APOEε4* allele not only increases the genetic susceptibility for AD in a dose-dependent manner but also influences the age of dementia onset, shifting to earlier onset in ε4 carriers, this might be one possibility.

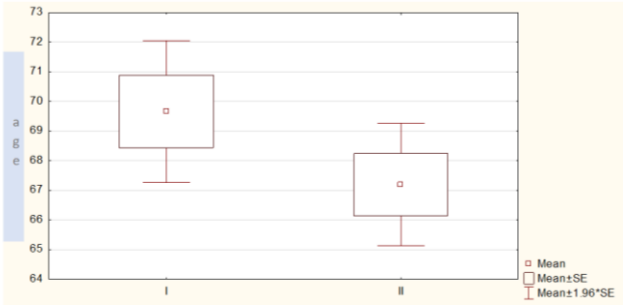

Figure 2: Mean age distribution among APOEε4 non-carriers-I and APOEε4 carriers-II

When we divided AD patients into two groups according to their *APOEε4* status, ε4 carriers and ε4 non-carriers, we found that average age of disease onset in the ε4 carrier group was 67.2 ± 8.3, and in the ε4 non-carrier group 69.7 ± 9.4. This confirms that the presence of *APOEε4* allele shifts towards earlier disease onset, though the difference is not statistically significant, $p > 0.05$ (t-test = 1.533270, p = 0.127864).

Discussion

In our AD patients study group, out of total 144 patients, 22.9% had the first symptom onset before the age of 65, that can be considered as EOAD, which is much higher than 5% as most studies report [1]. Out of those EOAD patients, *APOEε4* allele was present in 54.5% (in 94.4% as heterozygotes, and 5.6% as homozygotes).

Excluding the influence of *APOEε4* on the age of

onset we looked for the presence and influence of the modifiable risk factors.

When analyzed for the association of modifiable vascular risk factors (hypertension, diabetes mellitus and dyslipidemia) for AD in our study group compared to the control group, a statistically significant association was found for hypertension and $p < 0.05$ (Pearson Chi-square = 4.5302, $p = 0.033015$) and dyslipidemia $p < 0.05$ (Pearson Chi-square = 6.1103, $p = 0.013439$), but not for diabetes mellitus $p < 0.05$ (Pearson Chi-square = 0.0377, $p = 0.845696$). Having hypertension was found to be associated with 1.5-fold increased risk for developing AD [OR = 1.8767 (1.0479-3.3615)] and dyslipidemia was found to be associated with 2-fold increased risk for AD [OR = 2.2656 (1.1749-4.3688)] in our study.

Using multivariant logistic regression only the presence of *APOEε4* allele was confirmed to be a predictor for Alzheimer's disease.

Correlation among age of AD onset and hypertension, diabetes mellitus, obesity, dyslipidemia and physical activity was done, showing that only hypertension was significantly associated with earlier AD symptoms onset.

This confirms what previous studies reported by identifying hypertension, especially midlife hypertension to be one of the main modifiable risk factors for AD and as Winblad et al. suggest, pharmacological control of hypertension, implemented in middle-aged or younger old adults, might be effective in reducing the incidence of dementia [1]. Other population-based studies have shown that having multiple cardiovascular risk factors (smoking, hypertension, diabetes mellitus, hypercholesterolemia) in middle age or several years before dementia onset, increases the risk for developing AD [1], [9].

In conclusion, in our study group of 144 AD patients, a statistically significant association was found between the presences of *APOEε4* allele in AD group versus controls. The presence of *APOEε4* allele increases the risk for developing AD in a 3-fold manner and is the only confirmed to be a predictor for Alzheimer's disease. The average age of disease onset of 68.4 years could be considered earlier than the average age of AD onset worldwide. The presence of *APOEε4* allele shifts towards earlier disease onset, though the difference is not statistically significant.

Out of all the vascular risk factors analysed in this study, only hypertension and dyslipidemia were found to significantly increase the risk for developing AD and only the presence of hypertension influences the age of onset, shifting towards earlier disease onset.

Public awareness campaigns should be organised to influence general population knowledge about Alzheimer's disease, early recognition and the influence of modifiable vascular risk factors.

As the autosomal dominant inherited forms of EOAD are extremely rare, we should continue our research in evaluating potentially modifiable risk factors that influence the age of disease onset. Other design protocols might reveal other risk or lack of protective factors (such as higher levels of education, socialisation) that also influence the age of disease onset. Aggressive treatment of especially midlife hypertension could delay AD onset in genetically susceptible individuals that would aid more years to their life and more quality lifetime.

Further genetic research is needed especially in the EOAD subgroup to evaluate the presence of the three deterministic monogenic mutations in *APP, PSEN1* and *PSEN2* genes as potential genetic factors for EOAD, even though there is no family history that indicates autosomal dominant trait of transmitting. Only two decades ago, the diagnose of Alzheimer's disease in Macedonia was extremely rare and the term "sclerosis" was used to describe the dementia syndrome, which is why the general population does not report if someone from the family had forgetfulness, got lost (symptoms and signs that might be indicative for AD), which can mislead us in taking the family history. GWAS (Genome-wide associated study) might identify other than *APOE* genes, some of them specifically for geographic region or ethnicity, that might also influence genetic susceptibility, increase the individual risk for AD and decrease the age of symptoms onset.

In times when there is no etiological treatment that would stop or even slow down Alzheimer's disease progression, identifying individuals at risk and developing preventive strategies is more than necessary if we want to cut down the predicted numbers of affected population with Alzheimer's dementia till the year of 2050.

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