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ApoE polymorphism in Polish patients with Alzheimer's disease

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Short communication

Abstract. Alzheimer's disease is a genetically heterogeneous disorder of CNS. The presence of *APOE*-ε4 allele is known to increase the risk of early and late onset sporadic and late onset familial forms of AD. In various Western European countries, USA, Canada, Japan and Australia the allelic frequency ranges between 0.1- 0.18 in controls, and between 0.24- 0.52 in AD patients. In the present study on Polish population, we analyzed the frequency of *APOE*-ε4 allele in persons with Alzheimer's disease (AD). *APOE* genotypes were determined in 30 mild to moderate AD (83%) and mixed dementia (MIX, 17%), as well as in 11 nondemented first- degree relatives of AD (NDR), recruited from AD patient registry in Warsaw. Among the AD and MIX patients the *APOE*-ε4, ε3, ε2 allele frequency was 0.333, 0.65 and 0.017 respectively.

Key words: Alzheimer's disease, apolipoprotein E, polymorphism, Polish

Apolipoprotein E (ApoE) is lipoprotein present in plasma, where it has an important role in transport, metabolism and cellular recognition of serum lipoproproteins. In central nervous system it is produced by astrocytes, where it is thought to play a pivotal role in turnover of lipids or lipid soluble compounds (Mahley 1988). ApoE is produced in increased amounts in the CNS after experimental brain injury (Poirier et al. 1991). It has therefore been proposed that apoE may be involved in the pathogenesis of brain disorders.

Alzheimer's disease (AD) is a brain disorder, characterized by progressive dementia, accompanied by cortical neuronal loss associated with β-amyloid plaques, neurofibrillary tangles, and in most cases amyloid angiopathy (Tanzi et al. 1996). AD is genetically heterogeneous disease of familial and sporadic origin. Familial AD cases are associated with genes on chromosomes: 21 (mutations within exons 16 and 17 of amyloid precursor protein - APP), 14 and 1 (Tanzi et al. 1996). The gene for human apoE exists in three common allelic isoforms: *APOE*-ε2, *APOE*-ε3 and *APOE*-ε4. There is a convincing support evidence that a susceptibility gene *APOE*-ε4, located on chromosome 19 is a risk factor for sporadic and late- onset familial AD (Houlden et al. 1993, Mayeux et al. 1993, Strittmater et al. 1993). One copy of *APOE*-ε4 is associated with a moderately increased risk of AD (reported odds ratios range from 2·2 to 4·4) while two copies convey a high risk (odds ratios ranging from 5·1 to 17·9)(National Institute on Aging/ Alzheimer's Association Working group, 1996). The frequency of the *APOE*-ε4 allele in AD from living and autopsy series was shown to be between 30 and 40%, which is approximately three times that in general population (National Institute on Aging/Alzheimer's Association Working group 1996). *APOE*-ε4 allele has also been suggested to play a role in other disorders of CNS, undergoing with dementia: cortical Lewy body dementia (Betard et al. 1994), vascular dementia (Betard et al. 1994, Premkumar et al. 1994), cerebral amyloid angiopathy (Premkumar et al. 1994, Greenberg et al. 1995, Haan et al. 1995), Picks disease (Farrer et al. 1995), Creutzfeld-Jakob disease (Amouyel et al. 1994), posttraumatic encephalopathy (Nicoll et al. 1995), temporal lobe epilepsy (Kilpatrick et al. 1996) and schizophrenia (Harrington et al. 1995).

The role of ApoE in AD is still unknown. ApoE is a component of senile plaques and congophilic angiopathy, as well as neurofibrillary tangles (Namba et al. 1991, Wisniewski and Frangione 1992). Its capacity to bind β-amyloid peptides (Strittmatter et al. 1993, Wisniewski et al. 1993) and abnormally phosphorylated tau protein (Strittmatter et al. 1994) thus suggested that both β-amyloid deposition and PHF formation may be affected by apo E.

Recent studies on *APOE* polymorphism in Alzheimer's disease in several Western European countries, USA, Canada, Japan and Australia and lack of such of studies in Central Europe both prompted us to investigate an *APOE* polymorphism in Polish AD population. To further explore the role played by *APOE* genotype we have also studied the *APOE* polymorphism in patients with dementia of mixed origin (MIX), and first degree relatives of AD patients. The genotype analysis was done in Medical Academy and Medical Research Centre, Polish Academy of Science, Warsaw with approval of institutional review boards. All subjects underwent a standardized review and provided their informed consent at the AD patient registry, as well as in Department of Neurology, Medical Academy, Warsaw. Blood samples from AD patients and patients with dementias as well as AD relatives were collected in Department of Neurology, Medical Academy, Warsaw. Total number of genotyped samples included 41 individuals (16 M, 25 F). AD and mixed dementia (MIX) patients were diagnosed according to DSM-III-R and NINCDS- ADRDA criteria. The mean age at the diagnosis of dementia was 67.1 years (44-79), while the mean age of nondemented first degree (NDR) relatives was 54 years. *APOE* genotypes were determined in 26 mild to moderate AD (87%) and 4 mixed dementia (MIX,13%), as well as in 11 nondemented first- degree relatives of AD.

DNA was extracted from white blood cells, as previously described by Hixson et al. 1988. *APOE* genotyping was done using a method for restriction isotyping of Hixson and Vernier 1991. Leucocyte DNA was amplified by PCR in DNA Thermal Cycler (Perkin Elmer Cetus) using oligonucleotide primers: downstream primer (5'-ACAGAATTCGCCGGCCTGGTACACTGCCATGCCA-3') and upstream primer (5'-TCCAAGGAGCTGCAGGCGGCGCA-3'). Each amplification reaction contained 600 ng of leukocyte DNA, 75 pmols of each primer, 14 μl of 10% dimethyl sulphoxide, 3.5 units of Taq polymerase (Perkin Elmer Cetus), as well as nucleotide components in final volume of buffer 70 μl. Each reaction was heated at 94°C for 5 min., followed by 25 cycles of annealing (65°C for 30 s), extension (70°C for 90 s), denaturation (94°C for 30 s) and final extension at 70°C for 10 min. A 227 bp product of PCR amplification was digested for 5 h, at 37°C with 5 units of *HhaI* (New

England Biolabs). Each reaction mixture was loaded on 8.4%, 1.6 bis% polyacrylamide nondenaturating gel, subjected to electrophoresis for 4 h at constant voltage 80 V. After staining with ethidium bromide the digestion products were visualized under uV light, and their sizes compared to known size markers.

In this series of 30 patients diagnosed clinically as having mild to moderate AD or mixed dementia, 26 had AD and 4 had MIX. The results of *APOE* genotyping are shown in Table I. Among AD and MIX patients 18 had ε3/ε4 isotype, while we have found only one patient with ε4/4 isotype. No AD patients homozygous for ε2 allele were found, neither ε2/ε4 isotype was noted. The frequency of ε4 allele in AD group alone and AD combined with MIX was found to be higher (Table II), than that in the NDR group, which is in agreement with previously published observations (Anwar et al. 1993, Poirier at al., 1993, Strittmatter et al. 1993, Lehtimäki et al. 1995, Ibaretta et al. 1995). The observed *APOE*-ε4 allele frequency (33.3%) is in agreement with previously published data on late- onset patients with AD (Strittmatter et al. 1993, Ueki et al. 1993, Ibaretta et al. 1995, Lehtimäki et al. 1995, Schacchi et al. 1995). The frequency of *APOE*-ε2 allele in our AD combined with MIX was found to be lower than that in NDR group, however we are not able to discuss this problem more accurately at the present (only one patient with ε2/ε3 isotype).

We also noticed a lower frequency of *APOE*-ε3 allele in the AD group as compared to NDR individuals. NDR group studied at present was too small to allow us to draw statistically significant conclusions. However, *APOE* -

TABLE I

Results of *APOE* genotyping

APOE	AD *n* = 26 ~	MIX *n* = 4 ~	NDR n = 11 ~
ε4/4	1	0	0
ε3/4	15	3	4
ε2/4	0	0	0
ε3/3	9	1	5
ε2/3	1	0	2
ε2/2	0	0	0

AD, Alzheimer's disease; MIX, Mixed dementia; NDR, Nondemented first degree relatives; *n*, number of cases.

TABLE II

Results of *APOE* allele frequency

APOE allele	Allele frequency (%)		
	AD/MIX	AD	NDR
ε4	33.3	32.7	18.2
ε3	65	65.4	72.7
ε2	1.7	1.9	9.1

AD, Alzheimer's disease; AD/MIX, Alzheimer's disease and Mixed dementia; NDR, Nondemented first degree relatives.

ε4 frequencies in NDR group (18.2%) were higher than previously described for Caucasian population (10-16%) (Houlden et al. 1993, Mayeux et al. 1993, Saunders et al. 1993) and much higher than that from Spanish population (6%)(Ibaretta et al. 1995) or healthy French centenarians (5.2%)(Schächter et al. 1994). The mean age of our NDR individuals was lower than the age of AD or MIX patients. We speculate that some of these cases could potentially start suffering from AD in the future. However, a close follow up study is needed to confirm our clinical data. Usually frequency of pathologically confirmed diagnosis of AD is similar to that published series with necropsy results as the diagnostic reference standard (app. 85%) (Saunders et al., 1996). Large scale studies determining the role of *APOE* in Polish AD cohort and other dementias including aged matched controls are in progress.

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