Apolipoprotein E4 in Patients with Alzheimer's Disease in Slovak Region

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Summary

Many studies have found high frequency of ApoE e4 in patients with Alzheimer's disease (AD). The aim of this study was to asses the risk of AD in relation to genetic polymorphism of ApoE gene in Slovak region. Methods: Study of 34 patients, all subjects met the ICD-10 criteria for AD. The polymorphism of ApoE gene was investigated by polymerase chain reaction and digestion with Afl III. Results: The ApoE e4 allele was found in 44.1 % of patients (73 % of them with late-onset AD, 27 % with early-onset AD). The proportion of patients with non-ApoE e4 was 55.9 %. Conclusion: The frequency of patients with ApoE e4 in Slovak region is slink to the common results of western studies and confirms the role of ApoE e4 allele as a risk factor for AD (Fig. 2, Tab. 1, Ref. 40).

Key words: Alzheimer's disease, dementia, apolipoprotein E

Introduction

AD is considered to be a multifactorial disease with a strong genetic component. Several genetic factors are known to play important roles in the pathogenesis of this disease. One is the amyloid precursor protein (APP) gene on chromosome 21.

Investigation of large families with familial AD (FAD) led to the discovery of two additional AD genes, termed the presenilins. Presenilin-1 (Ps-1) is on chromosome 14. Mutations in this gene cause an early-onset AD. Presenilin-2 (PS-2) is on chromosome 1. Patients with mutations in these genes have elevated plasma levels of AB41-42 amyloid, suggesting a possible link between the presenilins and APP. Mutation in the chromosome-14 gene have thus far proved to be the most common cause of early-onset FAD, representing perhaps 70 % of this syndrome. Mutations in PS-1 tend to produce AD with an earlier age of onset and a shorter, more rapidly progress course than the disease caused by mutations in PS-2 (Small et al., 1997; Goate et al., 1991; Levy-Lahad et al., 1995; Schellenberg et al., 1991; Sherrington et al., 1995; Hardy, 1997; Clark and Goate, 1993; Cruts and Van Broeckhoven, 1998). The inheritance of a polymorphism in the gene encoding alfa-2 macroglobulin, a larger multifunctional protein that can act as a special kind of protease inhibitor, has been associated with increased risk of late-onset AD (Blacker et al., 1998).

A discovery of great importance has implicated the ApoE gene on chromosome 19 in the pathogenesis of late-onset familial and sporadic forms of AD (Strittmatter et al., 1993; Strittmatter and Roses, 1993; Poirier et al., 1993; Meyer et al., 1998). Apolipoproteins are protein components of lipoprotein particles. These macromolecular complexes carry lipids such as cholesterol and phospholipids from one cell to another within a tissue or between organs. Some apolipoproteins regulate extracelular enzymatic reactions related to lipid homeostasis whereas others are ligands for cell surface receptors that mediate lipoprotein uptake into cells and their subsequent metabolism (Rubinsztein, 1995; Rebeck et al., 1993).

ApoE is a component of several classes of plasma and cerebrospinal fluid lipoproteins and is believed to have an important role not only in reactive synaptogenesis by delivering lipids to remodelling and sprouting neurons in response to tissue injury, but also in physiological ongoing synaptic plasticity and maintenance of neuronal integrity as well as in cholinergic activity (Arendt et al., 1997; Poirier et al., 1999; Poirier, 1994).

Three alleles (e2, e3 and e4) at a single gene locus on the long arm of chromosome 19 code for the common isoforms of ApoE, namely, ApoE e2, ApoE e3 and ApoE e4. Allelic distribution in a typical elderly white population is approximately 8 % for e2, 78 % for e3 and 14 % for e4. The relative

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allelic frequencies in other ethnic groups such as black Americans, Hispanics and Japanese were reported to be similar among elderly populations (Farrer et al., 1997). But according to some findings, in Japan the relative predominance of vascular dementia over AD may be due to the rarity of apo E4 in this population (Gauthier et al., 1997).

The association between AD and ApoE genotype was first demonstrated in 1993 by Strittmatter el al. The group found a much higher frequency of the ApoE e4 allele in patients with late-onset AD compared with controls (50 % vs. 16 %). The ApoE e4 allele was found to be over represented in groups of both familial and sporadic cases of late-onset AD, which accounts for approximately 90 % of all AD cases. The e4 allele frequency was shown to be significantly higher (around threefold, or 40–50 %) in the AD population.

Estimates indicate that more than half of the susceptibility to AD is associated with the ApoE locus. Inheritance of one or two ApoE e4 alleles is associated with a dose-related higher risk and younger age of onset distribution of AD. Individuals with one ApoE e4 allele have a two-to fourfold increased risk of developing late-onset AD compared with those without the ApoE e4 allele whereas subjects who are homozygous for e4 have a five-to 34-fold increased risk (Burns et al., 1995; Corder, 1996). The effect of the least common e2 alleles appears to consist of decreasing the risk and increasing the age of onset to be protective (Roses, 1996; Mortimer and Graves, 1993). Studies of the linkage between apo E4 and AD have shown that carriers of the e4 allele have an increased risk of developing AD in an allelic dose-dependent manner (Roses, 1994; Bullido et al., 1998). The ApoE e4 genotype modulates the age of onset of the AD (Corder et al., 1993; Strittmatter et al., 1993). The inheritance of the e4 allele correlates with increased deposition of beta-amyloid in blood vessels and plaques in the cerebral cortex (Schmechel et al., 1993; Rebeck et al., 1993; Wisniewski and Frangione, 1992; Ingerslev, 1998). Furthermore, the density of cholinergic neurons in the basal forebrain, which represents the primary cholinergic input to these areas, was significantly reduced in e4 allele carriers compared with non-e4 AD patients or control subjects. The presence or absence of e4 alleles (in combination with gender) in a given subjects markedly affects the response to several antidementia drugs (Poirier et al., 1995; Poirier and Sévigny, 1998). An increased frequency of the ApoE e4 allele has also been found in patients with VaD (Frisoni et al., 1994; Marin et al., 1998; Shimano et al., 1989; Slooter et al., 1997), and Dementia with Lewy bodies (St Clair et al., 1994). Presence of ApoE e4 is also associated with risk of coronary heart disease and haemorrhagic stroke (Corder, 1996; Gauthier, 1996).

Our previous study (Shahpesandy et al., 2001) has found the ApoE e4 frequency in very small percent (17.4 %) of these patients. In this study we have extended the number of patients.

Methods and Materials

Study of 34 patients (female 21, male 13, mean age 76.310 years, age range 54–93), all subjects met the ICD-10 criteria for AD. Wechsler memory scale, Mini Mental State Exam, Hachinski score and Yesavage Depression Scale were used. Complete urine exam, biochemical exam of blood (Ca, Na, K, Mg, hepatal enzymes, urea, creatinin, glycemy), complex haematological exam, cortizol, lipidogram (cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and Triglycerides), and hormones of thyroid gland were investigated too. Specialists in Neurology, Internal medicine and Ophthalmology examined also all patients. Assessment by Computer Tomography was also used in some cases. All cases were Slovak people (Caucasians) from the region of Eastern Slovakia.

Genomic DNA was prepared from peripheral blood leukocytes by salted method. A 227bp region of DNA that spans ApoE polymorphic sites was amplified by polymerase chain reaction (PCR). The PCR was carried out in a MJ Research Thermal Cycler; after 3 min pre-treatment to 95°C, the reaction mixture was subjected to 35 cycles of 95°C for 30s, 68°C for 30 s, and 72°C for 1 min 30 s. This was followed by a final step at 72°C for 10 min. The resulting DNA fragments were digested with restriction endonuclease Afl III (37°C, overnight). The fragments after digestion were separated by electrophoresis through a 3 % agarose gel. The gels were stained with ethidium bromide and transiluminated with UV light. ApoE e4 allele differs from others alleles of ApoE gene by lacking the restriction sites for Afl III. The ApoE e4 allele was detected as a 227-bp fragment, the non ApoE e4 allele was detected as a 32- and 195-bp fragments (Fig. 1).

Results

Table 1 described the distribution of ApoE alleles in patients. Out of 34, in 15 patients (44.1 %) the ApoE e4 allele was found (female 9 mean age 78 years, range 60–93; male 6, mean age 71 years, range 61–81). 73 % of this group were with the late-onset AD (LOAD), mean age 80 years, range 68–93 (7 women mean age 82.4 years, range 68–93; and 4 men mean age 75.7 years, range 68–81). 27 % of them (female 2, male 2, mean age 62 years, range 60–65) were with the early-onset AD (EOAD). 55.9 % of patients (female 12, male 7, mean age 77.1 years, range 54–90) had no ApoE e4 allele. Three of them (female 2, male 1, mean age 59.3 years, range 54–63) with the EOAD, and 16 of this group (female 10, mean age 79 years, range 67–89, and male 6, mean age 83 years, range 73–90) with the LOAD. Homozygotes for ApoE e4 allele were not found in this group (Fig. 2).

Discussion

The frequency of ApoE e4 allele was found in 44 % of all patients, which is more than in our previous study was desc-

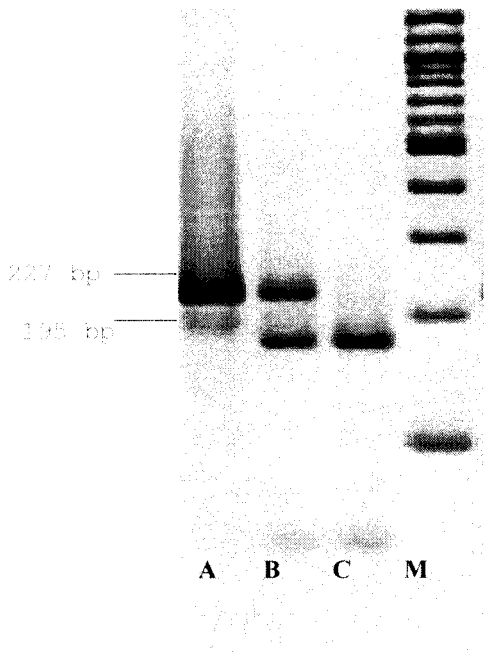

Figure 1. Separation of PCR-RFLP products for ApoE gene by agarose gel electrophoresis. M-100bp ladder, A-genotype (two alleles ApoE e4), B-genotype (one allele ApoE e4), C-genotype (non ApoE e4 alleles)

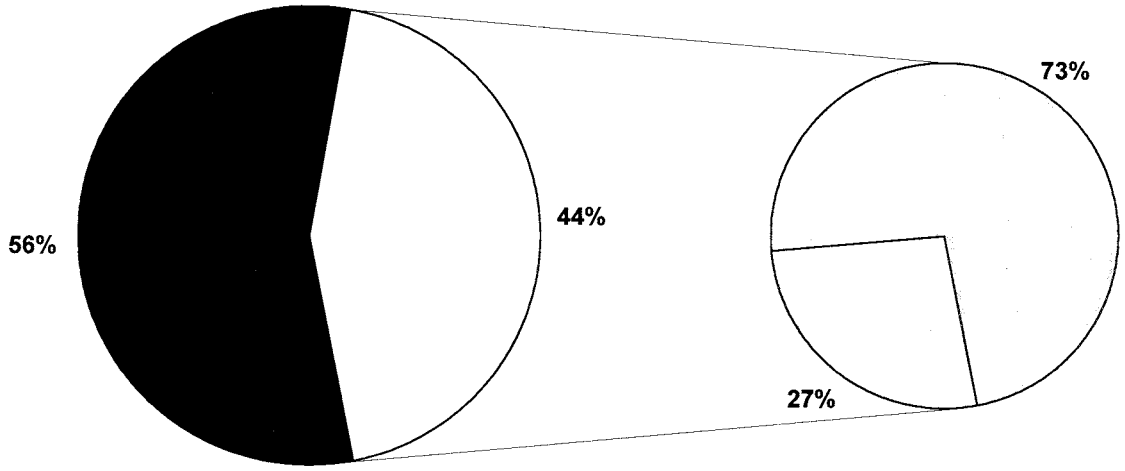

Figure 2. The frequency of ApoE e4

Table 1. Distribution of patients with and without ApoE e4 alleles

	ApoE		Non-ApoE e4	
Age of onset*	62.0±2.2	80.0±8.2	61.3±5.4	81.4±5.8
Male	2	4	1	6
Female	2	7	2	10
Total	15 (44.1 %)		19 (55.9 %)	

* mean±S.D.

ribed. These results slink to the common results of western studies.

ApoE has been shown to influence the rate of dementia in many diverse populations and has been unequivocally confirmed as the most important genetic influence on late-onset AD. The ApoE locus is not considered a causative gene, but rather a susceptibility or modifying factor as the presence of e4 allele is neither sufficient nor necessary for AD to develop. It is likely that many other genes will also contribute to risk either independently or in interaction with ApoE. All of these genes may also influence how individuals respond to environmental risk factors as divers as hypertension, head injury, and diet (Lovestone and Gauhtier, 2001).

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Do redakcie došlo 25.8.2002.