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Regulatory region single nucleotide polymorphisms of the apolipoprotein E gene as risk factors for Alzheimer's disease

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ABSTRACT

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by a complex aetiology. The ε4 allel of the apolipoprotein E gene (*APOE*) is the only confirmed genetic risk factor for the development of AD. In addition, polymorphisms at the promoter region of the *APOE* gene are assumed to modulate the susceptibility to AD by their different affinity to the transcription factors thus affecting the expression of the gene. In the presented study, we investigated the association between −491 A/T (rs449647), −427C/T, (rs769446) and −219 T/G (rs405509) single nucleotide polymorphisms (SNPs) of *APOE* gene and AD risk in the Polish population. We found that only the −491 T allele and −491 A/T genotype acted as protective factors against AD, whereas the −219 T/G heterozygosity increased risk for AD in *APOE* ε4 carriers but not in *APOE* ε4 non-carriers. What is more, haplotype frequency estimation showed significant positive for A-T-T-C-C and A-T-G-C-C haplotypes or negative for A-T-T-T-C and T-T-T-T-C haplotypes associations with AD. These results contribute to the evidence that *APOE* promoter polymorphisms modulate risk for AD.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by the presence of deposits such as amyloid plaques, neurofibrillary tangles (NFTs), and cerebral amyloid angiopathy (CAA) in the affected brain. The amyloid-β protein (Aβ) is the major constituent of both plaques and CAA. AD exists in either autosomal dominant familial or sporadic form. Mutations in three genes encoding β-amyloid precursor protein, presenilin-1 and presenilin-2 were identified to cause familial forms of AD. However, mutations in these three genes are responsible for only a small fraction of cases of AD, whereas majority of cases are not linked to any mutation. A non-Mendelian fashion of inheritance of this form indicates that it is a complex disease in which genetic as well as non-genetic factors are involved. Isoforms of apolipoprotein E (ApoE) were identified as a major risk factor for the sporadic form of AD. In addition to the main function of ApoE in the body that is the clearance of lipoproteins, it is also involved in the synaptic plasticity, cell signaling, lipid transport and metabolism, and neuroinflammation in the central nervous system [11,14].

ApoE is a polymorphic protein, with three isoforms named ApoE2, ApoE3, and ApoE4, encoded by three alleles (ε2, ε3, ε4) of the apolipoprotein E gene (*APOE*) as a result of two missense SNPs (rs429358 and rs7412) changing amino acids at residues 112 and 158, respectively. Numerous studies confirmed that presence of an ε4 allele increases the risk of both late and early onset forms of AD in an allele dose dependent manner and decreases the age of onset of the disease [8]. Conversely, bearing an ε2 allele confers a protection against the disease [7]. ApoE2 isoform has the most prolonged ability to prevent Aβ from converting into fibrillar forms, whereas ApoE4 isoform leads to greater Aβ deposition [9,12].

Recent reports suggest that, in addition to the qualitative effect of coding region polymorphisms, the level of *APOE* expression may be a crucial factor influencing the susceptibility to AD. It has been suggested that polymorphisms of the regulatory region of the *APOE* gene could affect *APOE* expression influencing Aβ accumulation level. Three polymorphic sites, −491 A/T (rs449647), -427C/T, (rs769446) and −219 T/G (rs405509), were found upstream of *APOE* coding region [2]. A number of studies have shown that the variants of *APOE* promoter are associated with an increased risk of AD, although the results are not completely conclusive [2,16]. To verify the association of *APOE*

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promoter polymorphisms with the development of AD in the Polish population we performed the case-control study. We analysed the allele, genotype, and haplotype frequencies of −219 T/G, −491 A/T, and -427C/T *APOE* SNPs among AD patients and healthy subjects in association with the susceptibility to AD.

2. Materials and methods

2.1. Subjects

The AD patients and controls recruited into our study were Caucasians originating from Poland. Diagnosis of probable AD (n = 273; mean age = 72.58 ± 8.99) were established according to the NINCDS-ADRDA criteria. The controls (n = 188; mean age = 68.01 ± 7.13) were defined as subjects without dementia symptoms and with integrity of their cognitive functions. Informed consent for research purposes was obtained from all AD and control subjects involved in the study. The study was conducted according to the Declaration of Helsinki and it was approved by a local Ethics Committee.

2.2. SNP genotyping

DNA was extracted from peripheral blood cells (PBMCs) according to standard procedures. *APOE* genotyping was performed using *Hha*I restriction enzyme as previously described [26]. The −491 A/T, -427C/T, and −219 T/G *APOE* SNPs were done by polymerase chain reaction (PCR) with sequence-specific primers followed by digestion with restriction enzyme. To investigate the −219 T/G polymorphism *APOE* fragment spanning nucleotides from -239 to -20 was amplified with 5′- AGA ATG GAG GAG GGT GTC CG and 5′- ACT TGT CCA ATT ATA GGG CTC C as forward and reverse primers [15]. The PCR product was subsequently digested with *Msp*I restriction enzyme. To genotype the -427C/T and −491 A/T polymorphisms nested amplification was performed. At first, PCR with the forward 5′- CAA GGT CAC ACA GCT GGC AAC and reverse 5′- TCC AAT CGA CGG CTA GCT ACC outer primers amplified the fragment extending from -1017 to +406 base pairs relative to the transcriptional start site. The next reaction with inner primers 5′- TGT TGG CCA GGC TGG TTT TAA (mismatched) and 5′- CTT CCT TTC CTG ACC CTG TCC amplified the fragment spanning -512 to -285 bases. The PCR products were digested with *Alu*I and *Dra*I enzymes for the -427C/T and the −491 A/T polymorphism, respectively [2]. All digested DNA fragments were separated and visualized by 3% agarose gel electrophoresis.

2.3. Statistical analysis

Significance in differences of allele and genotype distributions were calculated using $\chi 2$ or Fisher's exact (in case of tables with small expected frequencies) tests. The analysis was implemented in Statistica, version 12. The importance of over-representation of genotypes/alleles was expressed as odds ratio (OR) with 95% confidence intervals (CI). Haplotype frequencies were estimated and compared for the participant groups using SHEsis Online Version [22]. Linkage disequilibrium (LD) analysis was carried out using Haploview 4.2 in which D' and the LD r^2 coefficients between all pairs of the polymorphic loci were estimated [3].

3. Results

3.1. Allele and genotypic frequencies

All *APOE* SNPs in the control subjects were in the Hardy–Weinberg Equilibrium (HWE) at the adjusted significance level (P ≥ 0.05).

The distribution of the *APOE*ε2/ε3/ε4 genotypes and alleles in both AD and control groups is shown in Fig. 1. As expected the presence of the ε4 allele was a risk factor for AD (OR = 7.726; 95%CI = 4.404–13.744); P < 0.001), whereas the ε2 allele was strongly protective (OR = 0.495; 95%CI = 0.528 to 0.943; P = 0.029). Significant protective effect was also observed for the ε3 allele (OR = 0.306; 95%CI = 0.208 to 0.451; P < 0.001).

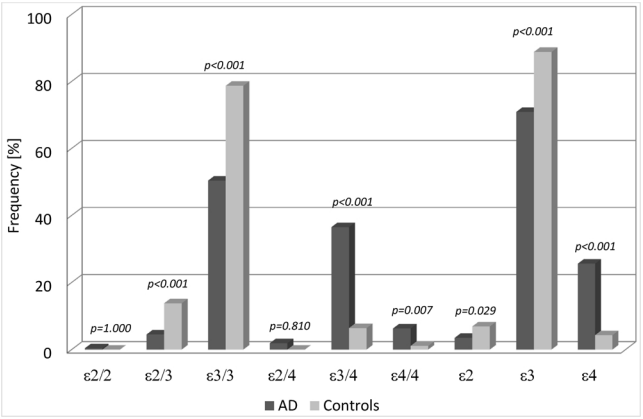

Fig. 1. *APOE* ε2/3/4 genotype and allele distributions in Alzheimer's disease and control population.

The distribution of the *APOE* promoter polymorphisms are shown in Table 1. The genotype and allele frequencies of −491 A/T SNP were significantly different between the AD patients and controls. The −491 T allele was significantly over-represented among the controls when compared with AD patients (OR = 0.693; 95%CI = 0.495 to 0.969; P = 0.03). The −491 A/T heterozygosity was observed significantly more frequently among the control group when compared with patients (OR = 0.526; 95%CI = 0.346 to 0.801; P = 0.002), whereas the −491 A/A homozygosity was less frequent in controls than in AD (OR = 1.752; 95%CI = 1.168–2.630; P = 0.006). There were no statistically significant differences in either genotypic or allelic distributions between the AD patients and control subjects for -427C/T and −219 T/G *APOE* SNPs.

In addition, our data set was split according to the ε4 allele status, resulting in two subgroups; those individuals who possess no ε4 allele and those who possess at least one copy of ε4 allele. As shown in Table 2 none of the SNPs of *APOE* promoter region showed significant difference in the genotype frequency between non-ε4 controls and non-ε4 AD patients. The −491 T/T genotype demonstrated increased frequency in AD group compared with controls (8.1% vs 4.6%) but the difference did not reach the level of statistical significance (P = 0.248). However, significantly higher frequency of the −491 T allele was observed among non-ε4 AD patients as compared to non-ε4 controls (35.7% vs 24.1%; OR = 1.173; 95%CI = 1.171–2.604; P = 0.005). Some differences in genotype distribution was observed among ε4 allele bearers. In ε4+ subjects the frequency of the −491 A/A genotype was increased in AD when compared with controls (79.2% vs 57.1%), however this difference did not reach statistical significance level (OR = 2.850; 95%CI = 0.787–10.205; P = 0.09). On the contrary, the −491 A/T heterozygosity was more frequently observed among controls than in AD patients (42.9% vs 20%; OR = 0.333; 95%CI = 0.093–1.212; P = 0.084). In the analysis stratified for the ε4 presence, frequency of the −219 T/G genotype was found to be significantly increased in ε4+ AD patients compared with controls (55.9% vs 0%; P < 0.0001). Moreover, the −219 T/T genotype occurred less frequently in AD group compared with controls (29.7% vs 85.7%; OR = 0.070; 95%CI = 0.010–0.359; P < 0.001). In consequence, the frequency of the −219 T allele was lower in AD than in controls (57.6% vs 85.7%), whereas the frequency of the −219 G allele was higher in AD than in controls (42.4 vs. 14.3%; OR = 4.412; 95%CI = 1.393–15.529; P = 0.004).