The Egyptian Journal of Medical Human Genetics (2016) 17, 185–189

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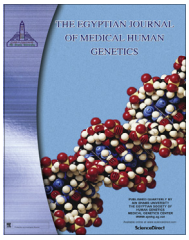

ORIGINAL ARTICLE

The association between (8390G > A) single nucleotide polymorphism in *APOE* gene with Alzheimer's and Parkinson disease

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Received 10 November 2015; accepted 24 November 2015
Available online 30 December 2015

KEYWORDS

Alzheimer's disease;
Parkinson's disease;
Polymorphism;
APOE;
rs121918398

Abstract Genetic susceptibility, is considered to be involved in neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD). Despite the fact that many susceptibility genes for AD and PD have been considered, the most probable genetic risk factor which has been taken into consideration is Apolipoprotein E genotype located on chromosome 19q, APOE is the gene widely considered to be a susceptibility gene for neurodegenerative diseases. This study is to investigate the association of APOE polymorphism with AD and PD. In this case control study we examined association of an APOE gene polymorphism (rs121918398) with AD and PD in Iranian population. The study included 100 AD patients, 100 PD patients and 150 healthy volunteers. An informed consent was obtained from all participants. Genomic DNA was extracted from peripheral blood leukocyte. Genotypes were determined by PCR and restriction fragment length polymorphism (RFLP) by Hha1 restriction enzyme. Sequencing of PCR products was carried out by Fazabiotech Company according to Sanger method using ABI 3730XL Capillary Sequencer. Statistical analysis was performed using the MedCalc program. The prevalence of genotype frequencies of the APOE A/A, A/G, G/G were 16%, 34% and 50% in AD subjects, 14%, 32%, 54% in PD patients and in healthy volunteers were 15%, 39% and 96% respectively. Statistical analysis showed no significant difference between genotype frequencies of AD and those of control subjects ($P < 0.05$). Moreover, according to statistical analysis, the genotype frequencies of APOE in PD subjects and control group did not significantly differ. This is the very first time that the association of this polymorphism (rs121918398) with AD is being reported nevertheless, there is no evidence that APOE variant is associated with PD. Accordingly, genotype alteration of A8390 > G can't be related to AD. So, this polymorphism plays no pathogenic role in the PD and AD patients in Iranian population.

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Peer review under responsibility of Ain Shams University.

http://dx.doi.org/10.1016/j.ejmhg.2015.11.003

1. Introduction

Dementia is the progressive decline in cognitive function due to damage of the brain, and is different from the slowing of cognitive function that is expected with normal aging [1]. Life expectancy has increased in last decades and health care improvements have contributed to people living longer. However, this has also contributed to increase in the number of people with chronic disabling diseases such as Alzheimer (AD) and Parkinson (PD). Several endogenous (genetic) and exogenous (environmental) factors contribute to the onset and/or development of these illnesses [2]. AD, the main form of dementia in aged people, is thought to account for 60–70% of cases. It is progressive, disabling and irreversible [3]. Clinic-wise, this disease is defined by progressive wane in memory, language, administrative function and other cognitional functions [4]. Progressive brain atrophy is observed in AD, with an inflammatory response of astrocytes and neurons, as well as deposition of neurofibrillary tangles (NFT) and amyloid plaques [5]. PD, the second most common neurodegenerative disorder after AD, is a chronic and progressive neurological disease, in which selective loss of dopaminergic neurons of the substantia nigra pars compacta (SNpc) is seen [6]. In addition to the neuronal loss, the existence of Lewy bodies (LB), in the surviving neurons, is the main neuropathological characteristic of PD, [7]. AD and PD have some clinical and neuropathological features in common [8].

In the central nervous system (CNS), glial cells produce *APOE*, and present it in high-density-like lipoproteins. APOE, binding to the amyloid-β (Aβ) peptide, interacts with different receptors which are members of the low-density lipoprotein receptor (LDLR) family [9]. In some neurons, in the case of brain injury, APOE levels increase drastically. This can affect neurite extension, Thus it is thought to play a key role in the protection and repair of neurons [10].

The genes *APOE2*, *APOE3* and *APOE4* (Three common isoforms of the protein) which are located within the *APOE* gene on chromosome 19, account for more than 99% of the variation of *APOE* [11]. Single nucleotide polymorphisms (SNPs), genetic variants that cause a difference in a single base pair in DNA, may lead to changes in a protein which might be important in a disease specific pathway [12]. SNPs are the most occurring polymorphisms of DNA sequence variations [13].

The human *APOE* gene contains many SNPs, spread across the gene [14]. The three most common SNPs resulting in changes in the coding sequence include: *APOE2* (cys112, cys158), *APOE3* (cys112, arg158), and *APOE4* (arg112, arg158) [15].

Although there have been many worldwide efforts on finding a relationship between *APOE* alleles and neurodegenerative disease, yet no report has been made on these SNPs [16], therefore, in this study, we investigated a polymorphism of *APOE* gene (8390G > A) and its association with AD and PD for the first time, which has been introduced as rs121918398. This polymorphism (APOE4) is pathogenic [13]. To our knowledge, so far, no study has been done on the effect of R292H mutation (*rs121918398*) on AD and PD development. So here we present the results of the investigation of association of this polymorphism with AD and PD and determine the prevalence of R292H mutation in Iranian population.

2. Subjects and methods

2.1. Study subject

In this case control study, 100 patients suffering from confirmed AD (74 females and 26 males), and 100 PD patients (46 females and 54 males) with the minimum age of 60 (mean 78.96 ± 10.99) were enrolled. The controls included 150 healthy people (93 females and 57 males) with the same ages (mean 77 ± 10.66). The sample selection was done randomly, whereas we know that the prevalence of neurodegenerative disease in females is more than males [17]. None of the controls had a clinical history of neurological diseases or had taken any drug related to nervous disorders. All donors gave their informed written consent prior to participation and completed a written questionnaire to give information related to their family history and ethnicity. The work has been carried out in accordance with The Code of Ethics of the World Medical Association (declaration of Helsinki) for experiments in humans that is approved by the local ethics committee of Guilan University. The predominant ethnic background of all groups were Iranian.

2.2. DNA extraction

DNA was extracted from peripheral blood lymphocytes using the Salting out DNA extraction method [18] and quality was confirmed on agarose gel 1%. All reactions were optimized to give clean ample quantities of DNA.

2.3. Polymerase chain reaction (PCR)

Amplification of a fragment containing this SNP was carried out by polymerase chain reaction (Eppendorf AG. 22331 hamburg). Primers were designed on the basis of the sequence data for APOE. PCR amplifications were performed in a final volume of 50 μl according to manufacturer's protocol (Bioflux PCR Kit) and an optimized PCR reaction was setup (Table 1).

2.4. Restriction fragment length polymorphism (RFLP)

The selected polymorphism (*rs121918398*) was detected by restriction analysis, performed on the PCR products, then analyzed on an agarose gel (1.7%). The substitution of the 8390G > A occurred and caused the conversion of arginine into histidine amino acid. There are three genotype modes (AA, AG, GG). A gap in the forward primer was introduced for distinguishing between the normal and mutant allele by *Hha1* restriction enzyme (Thermo scientific/Catalogue number: ER1851). The specific restriction enzyme and primers are summarized in Table 1.

The 89 and 35 bp fragments were obtained in G/G form, while in the A/G form 124, 89, 35 bp fragments and in the A/A form, a fragment of 124 bp without cutting site were obtained (Fig. 1).

Sequencing was performed to confirm PCR-RFLP analysis results. Sequencing of SNP was carried out by Fazabiotech Company (Tehran, Iran) according to the Sanger method using ABI 3730XL Capillary Sequencer. Sequencing results were compared with the sequence of normal APOE gene

(NC_000019.10) obtained from the NCBI website: http://www.ncbi.nlm.nih.gov (Fig. 2).

2.5. Statistical analysis

All statistical analyses were carried out using MedCalc software Version 12.7.0.0. In this study the genotype frequencies of control and patient groups were compared using the chi-square test. The *P*-value lower than 0.05 was set as statistically significant.

3. Results

This study investigated the polymorphism of a SNP loci 8390G > A in *APOE* gene in 100 AD patients, 100 PD patients and 150 control volunteers (males and females) using a PCR-based RFLP analysis. The distribution frequencies of the SNP loci in AD, PD patients and controls are listed in Table 2. Most studied participants were females, because the prevalence of neurodegenerative disease, especially AD, in females is significantly higher than males.

3.1. Genotype frequency in healthy volunteers, AD and PD patients

Among a total of 150 healthy persons, the genotype frequencies were 15, 39 and 96 and in AD patients were 16, 34, 50 (most homozygote E4 genotype were females) and in PD patients were 14, 32, 54 (most were males) for A/A, A/G and G/G respectively. The *P*-value was calculated by MedCalc software. As a result, no significant difference was observed in genotype frequencies among genotypes AA, AG, and GG in healthy, AD and PD patients ($P < 0.05$), except G/G form that its prevalence shows a significant difference in the control group compared with patient group.

3.2. Allele frequency in control, AD and PD patients

In the control group, the frequencies of allele A and allele G were computed as 23% and 77%, respectively. In addition, the frequencies of allele A and allele G were 33% and 67%, among AD patients, in PD patients the frequencies were 30% and 70% respectively. So no significant differences were observed in allelic frequencies between A and G in healthy and patient group (Table 2).

4. Discussion

In last decades, neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD), have been paid so much attention due to their high incidence worldwide [2]. These two disorders have some overlapping clinical and pathological features [19]. AD is diagnosed by cerebrovascular and neuronal dysfunctions resulting in a progressive decline in cognitive functions [20]. This multifactorial disease has some risk factors among which "older age" is the strongest one. This suggests that the biological processes of aging may play an important role in the pathogenesis of the disease [21]. PD, the second most prevalent neurodegenerative disorder after AD, is a neurological disorder. This condition is defined by the degeneration and progressive loss of neurons in the midbrain substantia nigra. This results in a reduction of dopamine in the striatum [22]. A potential link between *APOE* and PD traces back to the role of *APOE* in lipoprotein metabolism [8]. Apolipoprotein E (*APOE*) is a risk factor for Alzheimer's disease. It is proposed that it can change the risk of Parkinson's disease although it is considered not to significantly alter the risk of developing Parkinson's disease without Parkinson's disease dementia.

APOE, mapped on chromosome 19, is well documented as being a risk factor for the development of late onset Alzheimer's disease (LOAD) [23,24]. The 3 common *APOE* molecule

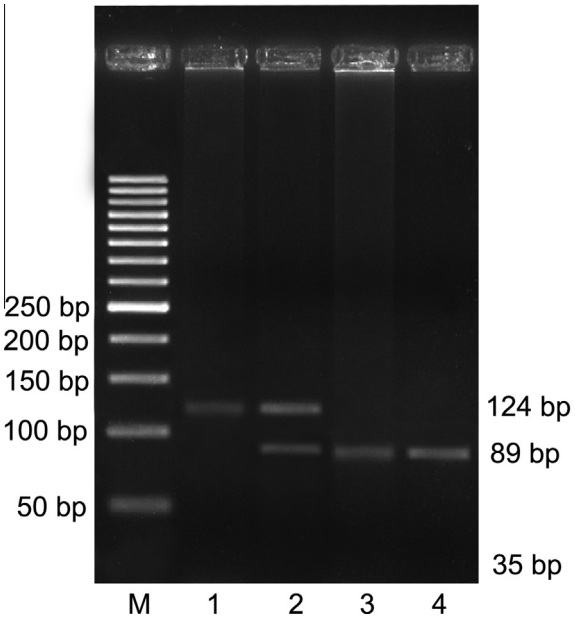

Figure 1 RFLP product. M line is 50 base pair (bp) molecular marker. In G/G form (3, 4), 89 and 35 bp, in A/G form (2), 124, 89, 35 bp and in A/A form (1), 124 bp fragment without cutting site were obtained. Because of the smaller size of 35 bp fragments, it moves through the gel faster and go out of the end, so we couldn't indicate the 35 bp band in this figure.

Table 1 PCR primers, annealing temperature, product size and fragments sizes after restriction treatment for *APOE*.

SNP ID	Amino acid change	Primer sequence	PCR product size	Restriction enzyme	Annealing temp	Mutant allele	Normal allele
rs121918398 (8390G > A)	A292H	5′ GAGCAGGCCCAGCAGATACG 3′ 5′ CAGCCTGCACCTTCTCCACC 3′	124 bp	Hha1	58	124	89, 35

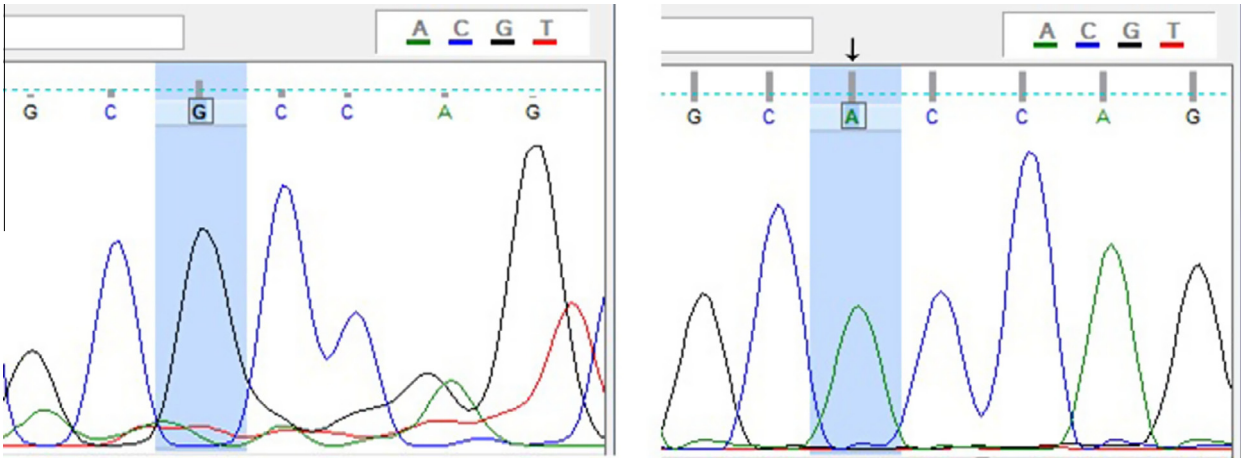

Figure 2 Direct sequencing of the PCR products of *APOE* gene. Arrow marks the sequence of 8390G > A polymorphisms.

Table 2 The distributions of allele frequencies of SNP 8390G > A in *APOE* gene in Alzheimer's and Parkinson patients and control group.

	AA	AG	GG
AD (100)	16	34	50
Control (150)	15	39	96
P value	0.16	0.17	0.02
Odds ratio	1.71	1.46	1.77
95% CI	0.80–3.64	0.84–2.54	1.06–2.97
PD (100)	14	32	54
Control (150)	15	39	96
P value	0.33	0.3	0.11
Odds ratio	0.68	1.33	1.51
95% CI	0.31–1.48	0.76–2.33	0.90–2.53

isoforms are encoded by variation in 3 common alleles (ε2, ε3, ε4). These alleles are specified by 2 single-nucleotide polymorphisms (SNPs) in exon 4 of the *APOE* gene [25]. Among the 88 identified SNPs in the *APOE* gene, 31 are not synonymous, 8 are coding synonymous, 33 are intronic and 3 are in untranslated region [26].

With regard to the fact that lifespan is increasing, neurodegenerative diseases are becoming of serious concern in these decades, particularly in developing countries. Accordingly, we investigated a polymorphism (*rs121918398*) in the *APOE* gene using the PCR-RFLP technique to study the substitution of arginine amino acid with histidine on codon 292. According to a previous study [13], *rs121918398* polymorphism is considered to be pathogenic and causing other sorts of diseases than neurodegenerative ones, but yet there has been no investigation on this polymorphism and its association with AD or PD development. In this survey, we investigated the genotype frequency of 150 healthy persons, 100 AD and 100 PD patients. The chi-square test result shows no significant difference between controls, AD and PD patient in homozygous and heterozygous groups ($P < 0.05$). Therefore, due to the impact of *APOE4* form on neurodegenerative diseases, this genotype plays no pathogenic role in the AD and PD. Thus, this polymorphism is not considered to be a risk factor for AD and PD in our studied population.

In a different survey, after ethnicity moderating, the *ε4* allele was found to be significantly associated with earlier age at onset ($P < 0.0001$) in the combined group. Among the Caucasians, the effect of the *ApoEε4*, on age of onset was significant ($P < 0.0001$). This was true for the African Americans as well ($P < 0.05$), but not significant in Koreans ($P = 0.43$) and in the smaller Hispanic ($P = 0.07$) group. The ε4 allele was found to be the most frequent one. This study shows that the impact of *APOE* polymorphism on age at onset of AD may vary among different ethnic groups [27].

Another study reports that *rs41377151* (located 10.9 kb away from *rs7412* which is one of the coding SNPs for *APOE* gene) was significantly associated with LOAD ($P = 3.29 \times 10^{-36}$) in the T Gen data set [28].

A different study showed that, among the 13 SNPs genotyped at < 40 kb from the *APOE4* polymorphism, 7 (including *APOE4*) had evidence of having association with AD ($P \leqslant 0.05$) [29].

Moreover, Sando et al. have reported the *APOE* allele frequencies in 376 AD patients and 561 healthy controls from central Norway (*rs7412* and *rs429358*). Carriers of the *APOE*-ε4 allele had an increased OR for developing AD. In both homozygous and heterozygous individuals, for every *APOE*-ε4 allele that the patient had, the age of onset in LOAD patients was significantly lowered (about three years). The *APOE*-ε2 allele did not affect the age at onset of AD and neither did this allele have any significant protective effect on the risk of AD [11].

Results of Multhammer et al. showed no correlation between combinations of alleles of APOE ε3 and APOE ε4 in their heterozygote and homozygote forms and prevalence of PD [30]. Neither did Peplonska et al. demonstrated any significant association at the single allele, genotype, or haplotype levels, concluding that *APOE* polymorphism is not a risk factor for Parkinson's disease in the Polish population [31].

Therefore according to the results of this study, it can be suggested that 8390G > A SNP of *APOE* gene plays no role in developing Alzheimer's and Parkinson's disease in Iranian population. Only G/G genotype illustrates a significant difference in the control group compared with AD patients ($P = 0.02$). It seems that the prevalence of this genotype in healthy population should be more than in patients. Accord-

ingly, future investigations on more individuals with AD and PD could be beneficial.

5. Conclusion

Among various polymorphisms of the *APOE* gene, some of which were relevant and/or irrelevant to AD and PD, this polymorphism (*rs121918398*) was investigated for the first time. It should be noted that some other polymorphisms of the various genes have been investigated in many studies about AD, PD etc. Since these diseases have multifactorial properties, broader studies should be conducted, within various races and generations; all environmental and genetic factors involved in AD and PD should be accurately studied.

Conflict of interest

The authors declare there is no conflict of interest.

Acknowledgment

We would like to express our gratitude to the owners and staff members of the Rasht, Lahidjan and Anzali Day Care Centres because of their assistance.

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