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Association between PSEN1 p.E318G Variant and APOE Polymorphism and Alzheimer Disease in Turkish Patients

Türk Hastalarda PSEN1 p.E318G Varyantı ile APOE Polimorfizmi ve Alzheimer Hastalığı Arasındaki İlişki

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Abstract

Objective: Mutations in the *Presenilin-1 (PSEN1)* gene have been associated with early-onset familial Alzheimer disease (AD) and these mutations usually exhibit full penetrance. However, the p.E318G variant located at exon 9 of *PSEN1* is an exception. This variant is also seen in non-demented controls other than patients with AD suggesting that it may be a rare polymorphism or a mutation with low penetrance. In addition, results from studies conducted in different populations investigating the role of p.E318G variant in AD were conflicting. In this study, we aimed to determine the frequency of the *PSEN1* p.E318G variant and *APOE* genotypes in a Turkish cohort and to investigate whether they were associated with the risk of AD.

Materials and Methods: The study included 217 patients with familial AD, 153 patients with sporadic AD, and 402 controls. The *PSEN1* p.E318G and *APOE* genotypes were determined using real-time polymerase chain reaction with hydrolysis probes.

Results: The p.E318G variant was found in five patients with familial AD, three patients with sporadic AD, and 11 control subjects. There was no significant difference in the distribution of the p.E318G variant between patients and controls in familial and sporadic forms. *APOE* ε*4* allele carriers had an increased risk for AD compared with non-carriers both in familial [odds ratio (OR): 3.67, 95% confidence interval (CI): (2.69-4.99); p<0.001] and sporadic cases [OR: 2.91, 95% CI: (2.06-4.10); p<0.001]. No significant difference was found in the distribution of the p.E318G variant with either the absence or presence of the *APOE* ε*4* allele.

Conclusion: Our results showed that *PSEN1* p.E318G variation, either alone or together with the *APOE* ε*4* allele, is not associated with AD risk in Turkish patients with AD. However, the *APOE* ε*4* allele constitutes a significant risk factor for AD both in familial and sporadic forms.

Keywords: Alzheimer disease, presenilin-1, E318G variant, *APOE* ε*4*

Öz

Amaç: *Presenilin-1 (PSEN1)* genindeki mutasyonlar, erken başlangıçlı ailevi Alzheimer hastalığı (AH) ile ilişkilendirilmiştir ve bu mutasyonlar çoğunlukla tam penetrans gösterir. Ancak *PSEN1* geninin 9 ekzonundaki p.E318G varyantı bunlar içinde bir istisnadır. p.E318G varyantının AH hastaları dışında demans olmayan kontrollerde de gösterilmesi, onun nadir bir polimorfizm ya da düşük penetranslı bir mutasyon olabileceğini düşündürmektedir. Bu varyantın AH'de patojenik rolü olup olmadığını araştıran ve birçok farklı popülasyonda gerçekleştirilen çalışmalarda çelişkili sonuçlar elde edilmiştir. Bu çalışmada, AH hastalarında ve kontrollerde *PSEN1* p.E318G varyantının ve *APOE* genotiplerinin sıklığını belirlemeyi ve Türk kohortunda AH riski ile ilişkili olup olmadıklarını araştırmayı amaçladık.

Gereç ve Yöntem: Çalışmaya 217 ailevi AH ve 153 sporadik AH hastası ve 402 kontrol dahil edildi. Hasta ve kontrollerin p.E318G ve *APOE* genotipleri hidroliz problar kullanılarak gerçek zamanlı polimeraz zincir reaksiyonu yöntemi ile belirlendi.

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Bulgular: p.E318G varyantı beş ailevi AH hastası, üç sporadik AH hastası, ve on bir kontrol örneğinde bulundu. Ailevi ve sporadik formlarda hastalar ve kontroller arasında p.E318G varyantının dağılımı açısından anlamlı bir farklılık görülmedi. *APOE ε4* allel taşıyıcılarının taşımayanlara göre AH riskinin hem ailesel [olasılık oranı (OO): 3,67, %95 güven aralığı (GA): 2,69-4,99, p<0,001] hem de sporadik olgularda (OO: 2,91, %95 GA: 2,06-4,10, p<0,001) artmış olduğu bulundu. *APOE ε4* allelin yokluğunda veya varlığında p.E318G varyantının dağılımında hastalar ve kontroller arasında anlamlı bir farklılık bulunmadı.

Sonuç: Çalışmamızın sonucunda elde edilen veriler Türk AH hastalarında *PSEN1* p.E318G varyasyonunun tek başına ya da *APOE ε4* aleli ile birlikte AH riski ile ilişkili olmadığını göstermiştir. Bununla birlikte, tek başına *APOE ε4* alelinin hem ailesel hem de sporadik formlarda AH için önemli bir risk faktörü olduğu görülmüştür.

Anahtar Kelimeler: Alzheimer hastalığı, presenilin-1, E318G varyasyonu, *APOE ε4*

Introduction

Alzheimer's disease (AD) is the most common cause of dementia seen in older adults. A common approach currently accepted according to the age of onset is to categorize AD into early-onset Alzheimer's disease [(EOAD); age at onset <65 years], and late-onset Alzheimer's disease [(LOAD; age at onset >65 years]. EOAD is mostly familial, and 10-15% of patients with familial EOAD exhibit an autosomal dominant transmission (1). Autosomal dominantly inherited EOAD has been associated with pathogenic variants in the *"Amyloid precursor protein (APP)", "Presenilin 1 (PSEN1)", and "Presenilin 2 (PSEN2)"* genes. Pathogenic variants in *PSEN1* gene are found in about 30-70% of familial EOAD cases and are associated with the most aggressive forms of the disease (2). The *PSEN1* gene encodes a transmembrane protein containing 467 amino acids, which cleaves APP into beta-amyloid peptides (Aβ) through gamma-secretase activity. Mutations in the *PSEN1* gene cause an increase in the Aβ42/Aβ40 ratio by impairing proteolytic cleavage of APP by gamma-secretase, and as a result, accumulation of Aβ plaques (3). To date, more than 300 mutations in *PSEN1* have been reported as pathogenic in the Human Gene Mutation Database (HGMD; www.hgmd.org).

One of the *PSEN1* variations considered to be associated with AD is the p.E318G variation (rs17125721) (4,5). This variation occurs due to an A to G transition at codon 318 in *PSEN1* exon 9 and leads to a non-conserved glutamic acid to glycine substitution. Results from studies conducted in different populations suggested that the pathogenicity of p.E318G in AD is controversial and it could be either an incompletely penetrant mutation or a rare polymorphism not associated with AD (6). It has been shown that the p.E318G variant was associated with high levels of total tau and phospho-tau in cerebrospinal fluid (4). Furthermore, coexistence of p.E318G variant with the *APOE* ε4 allele was associated with amyloid deposition and faster episodic memory decline (4). In the present study, our aim was to investigate the frequency of the p.E318G variant both in patients with sporadic and familial AD and controls and to evaluate its association with the *APOE* ε4 allele in a Turkish cohort.

Materials and Methods

Patients and Controls

The study population comprised 370 patients with AD and 402 controls without any history of major, systemic, psychiatric, and neurologic disease. The participants were recruited in the department of neurology, behavioral neurology and movement disorders unit, and underwent comprehensive clinical and neuropsychological examinations and neuroimaging. AD was diagnosed according to the National Institute of Neurological and Communicative Disorders and Stroke and AD (7). Approval was obtained from the Ethics Committee of İstanbul University-İstanbul Faculty of Medicine, Clinical Research Ethics Committee (decision no: 1209, date: 17/10/2016). The study was performed in line with the principles of the Declaration of Helsinki. Written and signed informed consent was obtained from all participants or legal guardians for subjects unable to consent.

Genotyping

Genomic DNA was isolated from peripheral blood leukocytes according to standard procedures. Screening of the *PSEN1* E318G variant was performed using quantitative real-time polymerase chain reaction (RT-qPCR) with a Taqman SNP Genotyping Assay (assay ID: C____589147_10, Thermo Fischer Scientific Inc.). A total of 10 µL qPCR reaction mix was composed of 0.5 µL TaqMan Genotyping Assay (Applied Biosystems), 5 µL LightCycler 480 Probes Master (Roche), 2.5 µL RNase-free water and 2 µL DNA (50 ng/µL). The qPCR was performed on a LightCycler480 system (Roche) under the following conditions: 95 °C 10 min, 45 cycles of 95 °C 15 s, and 60 °C 1 min. *APOE* genotypes were determined using RT-PCR with hydrolysis probes. The *APOE* RT amplifications included 10 min at 95 °C; 45 cycles of 10 sec at 95 °C; 30 sec at 56 °C and 1 sec at 72 °C. The end-point analysis was assessed using the LightCycler 480 genotyping software.

Statistical Analysis

Student's t-test was used for normally distributed continuous variables and Fisher's exact test for categorical variables. Maximum likelihood estimates of odds ratios (OR) and the 95% confidence intervals (CI) were calculated using binary logistic regression analysis. The SPSS software was used for all statistical analyses (IBM Corp., USA, version 21.0). P values below 0.05 were regarded as statistically significant.

Results

Patients with AD were divided into two groups: familial AD (at least one first-degree relative with a history of dementia) and sporadic AD (no family history of dementia). The characteristics of the study population are given in Table 1. A total of 370 patients with AD including 217 patients with familial (mean age: 64.6±10.7 years) and 153 patients with sporadic AD (mean age: 66.6±11.4 years).

As shown in Table 2, the p.E318G variant was found in five (2.3%) patients with familial AD and three (2%) with sporadic AD and in 11 (2.7%) control subjects. The p.E318G variant was detected only in the heterozygous state and no homozygous carriers were found. No significant difference in distribution of p.E318G was found for familial AD vs. controls (p=0.746) and sporadic AD vs controls (p=0.767).

In all groups, the distribution of allele frequencies and genotypes of *APOE* was significantly different (p<0.001) between patients and controls (Table 3). The ε3/ε4 and ε4/ε4 genotypes were found to be more common in the patient groups than in the controls. The *APOE* ε2, ε3, ε4 allele frequencies were 3.9%, 74.1%, and 22% in patients with familial AD and 2.6%, 78.6%, and 18.8% in patients with sporadic AD, and in the controls, the frequencies were found as 5%, 87.3% and 7.7%, respectively. Individuals with sporadic AD with at least one copy of *APOE* ε4 allele had a 2.91-fold increased risk [OR: 2.91, 95% CI: (2.06-4.10); p<0.001] for AD than individuals without the ε4 allele, and in patients with familial AD, risk increased up to 3.67 fold [OR: 3.67, 95% CI: (2.69-4.99); p<0.001].

To examine the possible interaction of the *APOE* ε4 allele and p.E318G variant, we investigated the distribution of p.E318G variant in ε4 allele carriers and non-carriers. In both the presence and absence of the *APOE* ε4 allele, no significant difference was found in the distribution of p.E318G variant between patients and controls in all groups (Table 4). However, there was a weak trend (p=0.268) for the coexistence of the *APOE* ε4 allele and the p.E318G variant in patients with familial AD. Of the five familial p.E318G carriers, three carried at least one copy of the *APOE* ε4 allele, but this trend was lacking in controls, as none of the p.E318G carriers were carrying the ε4 allele.

General characteristics of patients with the *PSEN1* p.E318G variant were given in Table 5. Six of the patients were diagnosed with EOAD, one with LOAD and one with atypical dementia. Of the three patients carrying *APOE* ε4 allele, two were homozygous and one was heterozygous for ε4 allele. The mean age at onset of patients was 56.2±11.2, range 36-76 years. The mean age of patients with the *PSEN1* p.E318G variant was 60.2±11 while controls with the p.E318G variant were younger (mean age ± standard deviation: 48.6±11.3) than the patients.

Discussion

In this study, we evaluated the frequency of the p.E318G variant and *APOE* genotypes and their contribution to AD in familial and sporadic forms. Also, we investigated whether there was an association between the p.E318G variant and the *APOE* ε4 allele.

The p.E318G variant is located on the hydrophilic loop between transmembrane domain VI and VII of PSEN1 protein (8). This region of the PSEN1 protein is less conserved between its homologues in other species and predicted to be functionally less important (9). The pathogenicity of the p.E318G variant is still under debate. In initial studies, it was considered as pathogenic in patients with EOAD (10,11); however, its presence in controls and no segregation with disease made its pathogenicity questionable (2,12,13,14,15,16,17,18). Even so, results from studies conducted in different populations investigating the role of p.E318G variant in AD were conflicting. Evidence from some case-control studies indicated p.E318G as a risk variant for patients with familial AD (2,12,13,18), but other studies found no significant association of p.E318G and AD (14,16,17,19). Therefore, the association between the *PSEN1* p.E318G variant and AD remains controversial.

The frequency of the p.E318G variant in our total patients with AD (2.1%) was lower than that described for European populations, actually it was the lowest reported to date (Dutch:

Table 1. General characteristics of patient groups and controls

	Patients		Controls
	Familial AD	Sporadic AD	
Number of individuals	217	153	402
Age (years)	64.6±10.7**	66.6±11.4**	57.6±13.1
Age at onset, years	60.4±10.4	62.3±11.3	-
Gender, n (%)			
Male	101 (46.5)	57 (37.3)	183 (45.5)
Female	116 (53.5)	96 (62.7)	219 (54.5)
MMSE score	18.9±7.7** (n=143)	16.8±7.8** (n=106)	28.9±2.1 (n=58)
GDS	10.3±5.9* (n=55)	11.2±6.2* (n=41)	7.19±6.3 (n=27)

Continuous variables are presented as mean ± standard deviation and dichotomous variables as percentage. A t-test was used for comparison of means and χ^2 test for percentages. AD: Alzheimer disease, MMSE: Mini-mental state examination, GDS: Geriatric Depression scale, **p<0.001 compared to controls, *p<0.05 compared to controls, standard deviation

Table 2. Distribution of PSEN1 E318G variant in patient groups and controls

	Patients (n=370)		Controls (n=402)
	Familial AD (n=217)	Sporadic AD (n=153)	
E318G+	5 (2.3)	3 (2)	11 (3.2)
E318G-	212 (97.7)	150 (98)	332 (96.8)
OR (95% CI)	0.84 (0.29-2.44)	0.71 (0.19-2.58)	-
p value	0.746[a]	0.767[b]	-

E318G+ refers to carrying heterozygous E318G variant, E318G- refers to carrying wild type E318G. PSEN1: Presenilin-1, AD: Alzheimer disease, OR: Odds ratio, CI: Confidence interval, [a]p value calculated by χ^2 test, [b]p value calculated by Fisher's exact test

Table 3. Distribution of allele and genotype frequencies of APOE in patient groups and controls

	Patients		Controls (n, %)
Genotype	**Familial (n, %)**	**Sporadic (n, %)**	
ε2/ε2	0 (0)	0 (0)	2 (0.5)
ε2/ε3	16 (7.4)	5 (3.3)	33 (8.2)
ε2/ε4	1 (0.5)	3 (2)	3 (0.7)
ε3/ε3	116 (53.7)	96 (63.2)	307 (76.6)
ε3/ε4	72 (33.3)	42 (27.6)	53 (13.2)
ε4/ε4	11 (5.1)	6 (3.9)	3 (0.7)
p value	<0.001	<0.001	-
Allele			
ε2	17 (3.9)	8 (2.6)	40 (5)
ε3	320 (74.1)	239 (78.6)	700 (87.3)
ε4	95 (22)	57 (18.8)	62 (7.7)
p value	<0.001	<0.001	-
APOE ε4 carrier status			
ε4 carrier	84 (38.7)	51 (33.3)	59 (14.7)
ε4 non-carrier	133 (61.3)	102 (66.7)	343 (85.3)
p value	<0.001	<0.001	-
OR (95% CI)	3.67 (2.69-4.99)	2.91 (2.06-4.10)	-

OR: Odds ratio, CI: Confidence interval

Table 4. Distribution of PSEN1 E318G variant in patient groups and controls stratified for APOE ε4 status

	Patients		Controls
	Sporadic AD	**Familial AD**	
APOE ε4-			
Number of individuals	102	133	343
E318G+	3 (2.9)	2 (1.5)	332 (96.8)
E318G-	99 (97.1)	131 (98.5)	11 (3.2)
p value	1.000	0.531	-
APOE ε4+			
Number of individuals	51	84	59
E318G+	0 (0)	3 (3.6)	0 (0)
E318G-	51 (100)	81 (96.4)	59 (100)
p value	-	0.268	-

E318G+ refers to carrying heterozygous E318G variant, E318G- refers to carrying wild type E318G. APOE ε4+ means carrying at least one copy of APOE ε4 allele, APOE ε4-means without carrying any copies of APOE ε4 allele. PSEN1: Presenilin-1, AD: Alzheimer disease, CI: Confidence interval, p value calculated by Fisher's exact test

2.7%, Italian: 3.3%, French: 3.5%, Finnish: 6.6%, Spanish: 6.7%, Australian: 3.6%, and Brazilian: 4%) (13,14,15,16,19). Similarly, the frequency of the variant in our control group (2.7%) was lower than the reported frequencies for European populations (Finnish: 6.8%, Spanish: 4.5%, Dutch: 4.1%, French: 4.1%, Polish: 4.0%) (14,15,16,17,19), but has similar frequency with the Australian population (2.2%) (12). Comparing the frequency of the p.E318G variant in patients with familial AD with those of other populations, our population has the lowest frequency (2.3%), but the frequency in patients with sporadic AD (2%) was consistent with the results from previous studies (2,12,13). Despite the larger number of patients included in our study, our results did not validate the previously reported significant associations of the p.E318G variant with AD. The lack of association between p.E318G variant and AD in our study is in agreement with previous studies that showed no significant associations either in patients with sporadic or familial AD in different populations (14,16,17,19). Combined with our results, these data suggested that the association between the p.E318G variant and AD might vary among different populations.

Table 5. General characteristics of patients with PSEN1 p.E318G variant						
ID	**Age**	**AAO**	**Sex**	**Family history**	**APOE status**	**MMSE**
Patient 1	61	59	Female	Familial	ε4/ε4	23
Patient 2	60	57	Female	Familial	ε3/ε3	19
Patient 3	67	63	Female	Sporadic	ε3/ε3	NA
Patient 4	56	53	Male	Sporadic	ε3/ε3	9
Patient 5	39	36	Male	Familial	ε3/ε3	NA
Patient 6	77	76	Female	Familial	ε3/ε4	12
Patient 7	66	53	Male	Familial	ε4/ε4	22
Patient 8	56	53	Female	Sporadic	ε3/ε3	9

PSEN1: Presenilin-1, NA: Not available, MMSE: Mini-mental state examination, AAO: Age at onset

The association between *APOE* gene and AD risk has been studied in a great number of populations. Studies showed that the frequencies of *APOE* ε2, ε3 and ε4 alleles varied between populations due to geographic locations and different ethnicities, but in most of them, the ε4 allele was considered as a risk factor for AD. In our study, the *APOE* ε allele frequencies in patients with AD and controls were very similar to those reported in the Turkish population (20,21,22,23) but were lower than in other populations (23). The low frequency of the *APOE* ε4 allele both in our study and previous studies is in line with the observation that ε4 frequency is low in Mediterranean countries. Our data confirm a clear association between *APOE* ε4 allele and AD; we found that the ε4 allele increased AD risk in patients with AD with a higher OR in patients with familial AD. The combined effect of *PSEN1* polymorphisms and *APOE* ε alleles on AD risk has been investigated previously in Turkish patients with LOAD; however, no significant difference was found (24).

The coexistence of the *APOE* ε4 allele and the p.E318G variant is associated with higher amyloid plaque deposition, faster episodic memory decline, and subsequent increased AD risk (4). In their study, Benitez et al. (4) showed that p.E318G variant modified AD risk in *APOE* ε4 carriers and increased the AD risk equally to ε4 homozygous in ε4 heterozygous; they showed that the *PSEN1* p.E318G carriers who also carried the *APOE* ε4 allele were at higher risk of developing AD than carriers of p.E318G variant alone, and had twice the risk of AD than those carrying the *APOE* ε4 allele alone (4). Therefore, to investigate the association of *APOE* ε4 allele and *PSEN1* p.E318G, we examined the distribution of p.E318G variant in *APOE* ε4 allele carriers. Although no significant association was found, our study highlighted the tendency for the coexistence of at least one copy of the *APOE* ε4 allele and the p.E318G variant in patients with familial AD.

Study Limitations

This study has limitations as follows. First, due to the small number of p.E318G carriers in this study, it is difficult to assess the significance of tendency for the coexistence of the *APOE* ε4 allele and the p.E318G variant in patients with familial AD. Further investigations in larger case-control series are necessary to confirm this association in the Turkish population because *PSEN1* p.E318G variant and *APOE* ε4 interaction is an important modifier of AD risk. Secondly, controls carrying the p.E318G variant were aged younger than 60 years and might later develop the disease; therefore, prospective follow-up examinations should be considered in controls. Another limitation of this study was that the control group was significantly younger than the patient group; this may not affect the results of the frequency of *PSEN1* p.E318G variation or *APOE* ε4 allele, but Mini-Mental State Examination scores may have been affected. Finally, another limitation was our relatively small sample size; the results of this study will be strengthened by studies with larger study groups.

Conclusion

In our study, we do not provide evidence for the association of the *PSEN1* p.E318G variant with AD risk and its interaction with the *APOE* ε4 allele. In addition, our results claim that the *APOE* ε4 allele is a significant risk factor in AD in the Turkish population. As far as we know, our study is the first to investigate the role of E318G variant in Turkish patients with AD. Therefore, further analyses in larger case-control cohorts are necessary to fully understand the effect of p.E318G on AD risk in the Turkish population. We suggest that special consideration should be taken in the interpretation of *PSEN1* p.E318G variant in AD, especially in familial forms.

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Ethics

Ethics Committee Approval: Approval was obtained from the Ethics Committee of İstanbul University-İstanbul Faculty of Medicine, Clinical Research Ethics Committee (decision no: 1209, date: 17/10/2016).

Informed Consent: Written and signed informed consent was obtained from all participants or legal guardians for subjects unable to consent.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: H.H., E.L., H.G., B.B., Concept: G.G., N.E.Ü., B.B., Design: G.G., N.E.Ü., R.A., Ç.D., Data Collection or Processing: G.G., H.H., E.L., H.G., R.A., Ç.D., Analysis or Interpretation: G.G., H.H., E.L., N.E.Ü., H.G., B.B.,

Literature Search: G.G., R.A., Ç.D., Writing: G.G.

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