

Article

Association of APOE Gene Polymorphism with Alzheimer's Disease and the Impact of Age, Gender, and Type 2 Diabetes Mellitus in Iraqi Patients

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Citation: Mohammad M. J. Association of APOE Gene Polymorphism with Alzheimer's Disease and the Impact of Age, Gender, and Type 2 Diabetes Mellitus in Iraqi Patients. American Journal of Biology and Natural Sciences 2026, 3(10), 107-112.

Received: 10th Jun 2026

Revised: 11th Aug 2026

Accepted: 20th Sep 2026

Published: 13th Oct 2026



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Abstract: The present study aimed to explore the linkage of APOE gene polymorphism with AD and to assess the effects of age, gender and type 2 Diabetes mellitus (T2DM) in Iraqi population. The study included 62 patients with AD and 48 control patients. In clinical results, the incidence of AD was observed to be higher in women than in men, higher with age, and T2DM was shown to be a significant factor in the progression of the disease. The molecular analysis was performed using Real-Time PCR for the APOE polymorphism (rs429358) and two alleles (T and C) and three genotypes (TT, TC and CC) were found within the study groups. The frequencies of the genotypes were TT (OR=0.46, 95% CI=0.25-0.91), TC (0.274) and CC (0.177) in the AD patients. Regarding allelic frequencies, the T allele frequency was 0.66 (OR=0.628, 95% CI=0.59–1.01), whereas the C allele frequency was 0.33 (OR=1.89, 95% CI=1.6–4.26). Based on these results, it can be concluded that the analyzed population is highly susceptible to AD because the APOE gene variants play an important role, in addition to other metabolic and demographic factors.

Keywords: Alzheimer's disease, Apolipoprotein E, Type 2 diabetes mellitus, Gene polymorphism.

Introduction

Alzheimer's disease (AD) is the most common type of dementia and the most common form of progressive neurodegenerative disease, accounting for about 60-70% of all clinical cases [1]. AD can be clinically divided into an early onset (age < 60) and a more common late onset form. There are many factors contributing to the etiology of AD, including multiple clinical comorbidities and lifestyle-related risk factors, which are interrelated and complex [2]. As the disease progresses, cognitive and behavioral problems worsen, such as profound disorientation, language difficulties, mood swings, a lack of motivation, and behavioral abnormalities, etc. [3]. This often leads to patients' isolation from family

and society as they lose their cognitive functions [4]. Slowly, simple bodily functions cease and ultimately death follows. The length of time after diagnosis ranges from three to twelve years, but is often variable between patients [5].

Although decades of intensive research have been undertaken, the exact mechanism of the disease underlying Alzheimer's disease still remains largely mysterious, with only 1–2% of cases being attributable to deterministic autosomal dominant genetic mutations [6]. The strongest and most well-established genetic risk factor for late-onset AD is the alleles of the Apolipoprotein E (APOE) gene, which encodes a major protein in mammals that plays a critical role in lipid metabolism [7].

The human APOE gene comes as part of the three major common polymorphic alleles, labeled $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$, according to two large coding area single nucleotide polymorphisms (SNPs). The APOE $\epsilon 3$ is the most common and is regarded as neutral with respect to risk, the relatively uncommon APOE $\epsilon 2$ allele is thought to have a protective effect against the disease [8]. Conversely, the APOE $\epsilon 4$ allele represents the primary genetic risk factor for late-onset AD. Compared to the wild-type homozygous genotype (APOE $\epsilon 3/\epsilon 3$), individuals carrying either heterozygous or homozygous copies of the APOE $\epsilon 4$ allele exhibit a significantly elevated risk of developing the disease [9]. Hence, this study aimed to explore this association between the enzyme polymorphism of the APOE gene (rs429358) and the susceptibility to AD among a particular group of Iraqis. Furthermore, the study was to estimate the importance of key demographic and metabolic parameters (age, sex and type 2 diabetes mellitus) in the examined population.

Materials and Methods

Study Population and Sample Collection

A total population of 110 persons - 62 patients clinically diagnosed as Alzheimer's disease and 48 healthy persons as the control group matched for age and sex - was used for this case control study. Participants' age ranged from 65 years to 85 years. A 4 mL sample from the periphery was drawn from each of the participants. The sample was collected in an EDTA tube, which was then used to extract genomic DNA for molecular analysis, and the remaining plasma in a plain tube for biochemical analysis.

DNA Extraction

Whole blood samples were used for isolation of genomic DNA with a commercially available DNA extraction kit following manufacturer's instructions.

Estimation of DNA Concentration and Purity

Extraction genomic DNA concentration and purity were assessed by the help of Nano-Spectrophotometer. Two μL was used in each case for the extracted DNA sample. Optical density (OD) ratio of A 260 /A 280 was used to calculate the purity of DNA. A ratio generally between 1.8 and 2 was accepted as acceptable for the downstream molecular analysis, while values below 1.8 suggested contamination with the residual proteins or organic solvents from the molecular analysis.

Real Time Polymerase Chain reaction (RT-PCR)

Polymorphisms within the APOE gene were studied in both patient and control groups using the Real-Time Quantitative PCR technique (qRT-PCR) in the same region (rs429358) studied in the other experiments. This molecular analysis took advantage of a newly developed fluorogenic TaqMan probe assay originally using allele-specific oligonucleotide probes labeled with different fluorescent reporter dyes to increase the level of allelic discrimination and the sensitivity of the allelic discrimination reaction [10, 11]. Each of the qPCR assays was summarized in Table 1 for the reaction volumes and components.

Table 1. Reaction mixture components for Real-Time PCR amplification

Component	Final volume(25 μL)
2X TaqMan® Master	12.50 μL
20X Assay Working	1.25 μL
Nuclease-free	-

DNA Sample Volume	11.25 µL
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Preparing of Custom SNPs Genotyping Solution

The allelic variations of the APOE gene at rs429358 were differentiated using TaqMan assay. Table 2 lists the context sequence and product properties of the specific site.

Table 2. Context sequence and assay parameters for the APOE (rs429358) locus

SNP	Catalog	Assay ID	Sequence
rs (429358)	4351379	C-3084793-20	GCTGGGCGCGGACATGGAGGACGTG[C/T]G CGGCCGCCTGGTGCAGTACCGCGG

Results

Clinical and Biochemical Characteristics

Details of the study groups are provided in demographic and biochemical profiles (Table 3). This was confirmed in the clinical measurements, with a significantly higher mean systolic blood pressure (SBP) in the Alzheimer's disease group than in the healthy control group (148.26±15.94 mmHg versus 142.73±16.43 mmHg, respectively, $p<0.001$). Mean diastolic blood pressure (DBP), however, failed to show a statistically significant difference between the groups; and 83.97±11.12 mmHg vs. 82.02±10.41 mmHg ($p=0.35$). Moreover, fasting blood glucose was significantly increased in AD patients (210.20±5.84 mg/dL) compared to the control group (124.66±3.26 mg/dL, $p=0.001$), reflecting a high degree of metabolic dysregulation in these patients.

Table 3. Baseline demographic, clinical, and biochemical characteristics of the study groups

Parameters		The group		p-value
		Control Mean ± SE (48)	Patient Mean ± SE (62)	
Age		(65 – 85) year		
Gender	Male	20	22	
	Female	28	40	
Systolic blood pressure (mmHg)		142.73 ± 16.43	148.26 ± 15.94	<0.001
Diastolic blood pressure (mmHg)		82.02 ± 10.41	83.97 ± 11.12	0.35
Glucose (mg/dl)		124.66 ± 3.26 b	210.20 ± 5.84 a	0.001*

Results Molecular study

The results of the ApoE gene analysis showed that there are three genotypes: TT, TC, and CC. The letters in the genotype indicate whether the individuals have the normal homozygous form, heterozygous form, or homozygous mutant form of the gene. The letters within the genotype represent the normal homozygous form, heterozygous form, and normal homozygous mutant form of the gene. The frequencies of the three genotypes (TT, TC and CC) observed in patients with Alzheimer's disease and controls are summarized; Table 4. The TT genotype recorded the highest frequency (0.548) with an odds ratio (OR) of 0.46 (95% CI: 0.25–0.91). The TC genotype had a frequency of 0.274 with an OR of 1.41 (95% CI: 0.59–0.96). In the patient group with Alzheimer's disease (AD), the CC genotype was present in 2.9 times more frequently (OR=2.9, 95% CI: 1.15–10.37) with a frequency of 0.177. The

frequencies of TT and TC and CC genotypes in the control group were 0.333, 0.500 and 0.166 respectively.

Table 4. Shows the frequencies of the three genotypes

Genotypes		Groups		
		TT	TC	CC
Patient (n=62)	Observed(Frequency)	34(0.548)	17(0.274)	11(0.177)
	Expected (Frequency)	28 (0.451)	27 (0.435)	7 (0.112)
Control (n=48)	Observed(Frequency)	16(0.333)	24(0.5)	8(0.166)
	Expected (Frequency)	19 (0.395)	26 (0.541)	3 (0.062)
p-value		0.33	0.63	0.29
Chi-Square (X ²)		5.746	0.357	4.265
OR		0.46	1.41	2.9
95% CI		0.25 – 0.91	0.59 – 0.96	1.15 – 10.37

Genotype frequencies of ApoE in Alzheimer patients and in the control group. Table 5 lists the number of the C and T alleles of the gene in persons with Alzheimer's disease and normal controls. Patients were seen to have an OR of 0.628 (95% CI: 0.59–1.01) and a T-allele frequency of 0.66, while those in the control group had an OR of 0.69 (95% CI: 0.62–1.00). The data also revealed a frequency of the C allele of 0.33 in patients and 0.30 in the control group with an OR of 1.89 (95% CI: 1.6–4.26).

Table 5. Allelic frequencies of the APOE (rs429358) polymorphism

rs429358			
Allele Frequency		T	C
Patient (n=62)	Number (Frequency)	82 (0.66)	42 (0.33)
Control (n=48)	Number (Frequency)	26 (0.69)	22(0.30)
Chi-Square (X ²)		4.078	
p-value		0.074	
OR		0.628	1.89
95% CI		0.59-1.01	1.6 – 4.26

Discussion

This study highlights the complexity of the Alzheimer's disease's onset, and the interplay of genetic risk and demographic factors as well as metabolic comorbidities. In our cohort, there was a higher number of women than men with AD. This agrees with significant epidemiological evidence showing that gender can have a significant impact on cognitive resilience, for instance when women hit menopause, endogenous estrogen levels decrease, which can seriously weaken resilience [15]. Additionally, the longer lifespan of women is a worldwide phenomenon, which contributes to their having higher exposure to age-related neurodegenerative stresses throughout their lifetime [16,17]. Age continues to be the most important unmodifiable risk factor, as there is a two-fold increase every five years after age 65 [16].

In addition to demographic factors, chronic metabolic diseases, such as Type 2 Diabetes Mellitus (T2DM) and systemic hypertension also appeared to be good correlates of AD in our study. AD patients were characterized by significant hyperglycemia and hypertension. Systolic blood pressure was significantly raised and hyperglycemia was significantly increased in AD patients. Over time, glucose dysregulation contributes to the blood-brain barrier becoming leaky, to cerebral microvascular disease and causes a loss in metabolic efficiency of neurons, so that AD is considered a complex metabolic brain disease [18,19]. In addition, a systematic exercise program or a specific change in diet has been shown to limit A β deposition, systemic neuroinflammation, and maintenance of cognitive functions [20,21], but these neuroprotective effects seem to be associated with a strong genetic disposition for the individual in terms of APOE genes. We analysed the APOE rs429358 at the genetic level. The increased risk of AD with the mutant C allele of the ϵ 4 allele was nearly twofold (OR=1.89), which is the variant that structurally defines the presence of the high-risk ϵ 4 isoform. It has been established that the APOE ϵ 4 isoform alters normal physiological lipid-binding and cholesterol transport inside the central nervous system, leading to neuro-degeneration at an earlier age, and that the mmLOS rapidly induces shortening of telomeres in a similar manner to premature cellular senescence [22-26].

Conclusion

Finally, this research substantiates the clinical value of using APOE gene polymorphisms as potential molecular markers for the estimation of susceptibility for developing Alzheimer's disease in the Iraqi population, adding to clinical risk factors such as older age, female sex and comorbid type 2 diabetes. The genetic distributions, however, should be taken with a grain of salt because of the small sample size of the current cohorts. A large, multi-center prospective study is highly encouraged to help define and fully characterise the exact genetic structure of Alzheimer's Disease in Iraq, including the gene-gene, and gene environment interactions.

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