Apolipoprotein E genotyping variations and their influence on cognitive outcome among idiopathic generalized epileptic patients

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Background/aim
Epilepsy is a brain disorder affecting individuals of all ages. It is one of the most common neurological disorders, affecting about 50 million people globally. The study objective was to study the different genotypes of Apolipoprotein E (*APOE*) gene among adult idiopathic generalized epileptic patients and to assess their influence on cognitive functions of those patients.

Patients and methods
Forty adult patients with idiopathic generalized epilepsy (IGE) and 20 healthy controls were enrolled in this study from Kafrelsheikh Governorate, Egypt. Patients were recruited from the Neurology Department at Kafrelsheikh University Hospital. Neuropsychological evaluation was conducted to all participants. Genotyping of the *APOE* gene polymorphisms (rs429358 and rs7412) was then carried out using both restriction fragments length polymorphism and Sanger sequencing to evaluate the correlation between the genotype and cognitive functions of patients with IGE.

Results
Most of the patients and controls showed the wild type genotype ε3/ε3 of both polymorphisms of *APOE* gene; rs429358 and rs7412. Only six patients and one control had different genotypes other than the wild type. The study showed that a highly significant association ($P<0.001$) between IGE and cognitive impairment especially attention, memory, and fluency. Meanwhile, no statistically significant differences were found in distribution of Addenbrooke score and parameters according to *APOE* genotype distribution in patients' group, while in controls there was no significant difference in distribution of attention and visuospatial processing parameters according to genotype distribution. Yet, there was a significant increase in Addenbrooke, memory, fluency, and language processing in ε3/ε3 genotype compared to ε3/ε4 genotype, (P=0.000, 0.007, 0.016, and 0.000, respectively).

Conclusion
This study supports the evidence of a distinct cognitive profile in patients with epilepsy regardless the *APOE* gene genotype.

Keywords:
Apolipoprotein E gene, cognitive profile, genotyping, idiopathic epilepsy, polymorphisms

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Introduction

Epilepsy is a brain disorder in which neurobiological, social, cognitive, and psychological issues are present. It affects patients of all ages and sexes, with an estimated incidence of 700 per 100 000 people and a prevalence of 50 per 100 000 people, respectively [1]. Around 50 million epileptics in the world and 80% of them live in low-income and middle-income countries [2].

Although epilepsy's epidemiological data are scarce in Egypt, epilepsy has a comparatively high prevalence and incidence in Upper Egypt. According to a community-based survey carried out in the Qena governorate, the incidence rate of epilepsy was 123/100 000, and the active prevalence rate was 2.12/1000 [3]. Uncontrolled epileptic seizures are reported to reduce the patients' quality of life impacting both their psychosocial functioning and physical health [4].

Epileptic patients have a high risk of cognitive impairment, which hinders their ability to succeed in school and life. There is a known IQ and achievement gap in nearly half of children with epilepsy in addition to a higher frequency of low IQ. Regression in cognitive ability can also occur in adults with chronic epilepsy [5].

According to the International League against Epilepsy (ILAE) 2017, there are four categories for epilepsy: focal; generalized; combination generalized and focal; or unknown [6]. In more than 50% of the cases, the cause of the seizures is unknown. These epilepsies were labeled "idiopathic" in the 1989 categorization of seizures [7]. Idiopathic generalized epilepsy (IGE) is defined as the absence of symptoms or signs internally, and structural abnormalities of the brain on MRI which rules out the majority of the etiological groups. Accordingly, generalized interictal spike-and-wave discharges and normal background electroencephalogram (EEG) activity are two characteristics of IGEs [8].

Idiopathic epilepsies are significantly influenced by genetic factors. More than 20 genes are known to be highly susceptible to idiopathic epilepsies [7]. The pathogenesis of idiopathic epilepsies is influenced by ion channel genes and a few nonion channel genes [9]. Wang and colleagues compared their results with those from the Human Gene Mutation Database (HGMD) and the Epilepsy Gene databases, they discovered 247more genes, and there are an additional 37 genes. These genes are said to have a potential link to epilepsy. In most instances, their connections to epilepsy call for additional investigation [10].

The Apolipoprotein E (*APOE*) gene in humans, which codes for a 299 amino acid protein is mapped to chromosome 19 at location q13.32 [11]. *APOE* is involved in suppression of platelet aggregation, tissue repair, immunoregulation, pathogenic, and physiological processes of senile dementia, cell proliferation inhibition, and the process of the nervous system's normal growth and healing after injury. It also plays a role in the nervous system development, immunoregulation of lipids, cellular regulation, and lipid activities during the repair and regeneration of damaged nerve cells [12]. Two common single nucleotide polymorphisms (SNPs) were reported to be naturally occurring in the *APOE* gene. The combination of variations at two SNPs (rs429358 and rs7412) defines three main *APOE* alleles namely ε2, ε3, and ε4. Since there are two copies of each allele, these three alleles result in six potential genotypes: ε2/ε2, ε2/ε3, ε2/ε4, ε3/ε3, ε3/ε4, and ε4/ε4 [13]. The two SNPs affected the Cysteine amino acid number 112 and Arginine at position 158. The most common genotype is *APOE* ε3, where position 112 is Cysteine and Arginine is at 158, is considered the wild type. In case both positions carry Cysteine, they create the *APOE* ε2 genotype, but if both positions carry Arginine, then the genotype changes to *APOE* ε4 [14].

Studies have shown how *APOE* different genotypes contributes to the development of late-onset Alzheimer disease, although it is still unknown how it is related to cognitive impairment. Polymorphism in the *APOE* gene is an important risk factor for late-onset Alzheimer disease, which usually appears beyond the age of 65 [15]. It has been established that the *APOE* gene poses a significant genetic risk for AD and other neurodegenerative illnesses. It seems sense to investigate the relationship between the *APOE* gene and epilepsy risk, given that individuals with certain disorders (such AD) have an elevated chance of developing epilepsy [16]. Here, the present study aim to evaluate the different genotypes of *APOE* gene (rs429358 and rs7412 polymorphisms) among adult idiopathic generalized epileptic patients and to assess their influence on cognitive functions of those patients.

Patients and methods

Patients

This cross-sectional study including 60 Egyptian participants from Kafrelsheikh University Hospital, Egypt, with matched age and sex. Their age ranges from 15 to 60 years. They were recruited during the period of 2021–2023. The present study was conducted by collaboration between the Neurology Department at Kafrelsheikh University Hospital, National Research Centre (NRC), and Biochemistry Department at Faculty of Pharmacy (Girls), Al-Azhar University in Egypt.

Study design

Sixty participants were recruited and split into two groups: the epilepsy-affected individuals enrolled 40 patients as group 1 and 20 healthy controls as group 2. Both patients and controls were prospectively referred from the Neurology Department at Kafrelsheikh University Hospital.

Inclusion criteria

Patients were selected according to 2017 ILAE Classification guidelines. Clinical evaluation confirms their diagnosis as IGE and proves the absence of signs and symptoms of other etiological groups of epilepsy. Their MRI shows no evidence of structural lesions. Their EEG excludes symptomatic epilepsy. Regarding control group the inclusion criteria including healthy participants were chosen for the study's control group with matched age and sex with epileptic group.

Exclusion criteria

Patients under the age of 15, patients with symptomatic epilepsies, static encephalopathy,

developmental delay, central nervous system infection history were excluded. Patients with liver, autoimmune, renal, and inflammatory diseases histories, allergic reaction, diabetes, immune deficiency disorder, psychiatric illness, cancer, sever cognitive impairment, clinical disorder that causes cognitive impairment or sever electrolyte imbalances also were excluded.

Ethical approval

The present study was conducted with the Code of Ethics of the World Medical Association, according to the principles expressed in the Declaration of Helsinki. The study design was approved by the Ethical Committee of the Faculty of Pharmacy (Girls), Al-Azhar University, with approval number 33-442. A written informed consent was acquired from each individual involved or their guardians, prior their inclusion in the study.

Methods

The following procedures were applied to all patients and controls.

Clinical evaluation

Detailed clinical evaluation with a focus on the following: sex, age, a family history of epilepsy, age at onset, epilepsy duration, seizure frequency, history of febrile convulsions, antiepileptic medication, and drug adherence. Drug adherence is known through "Morisky 4-item Medication Adherence Scale" [17]. Other investigations including EEG and brain MRIs were evaluated to exclude symptomatic epilepsy.

Neuropsychological profile

The patients and control groups underwent a neuropsychological assessment using an Addenbrooke test. It measures several areas of cognition in a short amount of time. It measures five subdomains of cognition: verbal fluency, language, visuospatial skills, attention and orientation, and memory. Higher scores indicate stronger cognitive functioning. The total score is out of 100 (18 points for attention, 26 for memory, 14 for fluency, 26 for language, 16 for visuospatial processing). It takes 15–20 min to finish Addenbrooke's Cognitive Examination-III [18].

A score of 88 or more is deemed, a score below 83 is abnormal, and a score between 83 and 87 is inconclusive. The result was evaluated in the context of the patient's complete medical history and examination [19]. Because it has excellent sensitivity, specificity, and positive and negative predictive values, we employed the validated Egyptian-Arabic ACE-III [18].

APOE genotypes analysis

DNA extraction

From each participant, 3 ml of venous blood were withdrawn into a tube containing K_2EDTA as an anticoagulant, which was subsequently kept at 4°C. Using a Genejet Whole Blood genomic DNA purification kit (Thermo Scientific, Waltham, MA USA), genomic DNA was extracted from peripheral blood leukocytes in accordance with manufacturer instructions. This kit uses the column purification method. DNA samples were then stored in the temperature of −20°C. The extracted DNA quality and quantity was measured using NanoDrop 200 spectrophotometer (Thermo Scientific).

PCR

PCR was carried out using specific primers which designed to amplify the exon number 4 carrying both SNPs. The PCR mixture consisted of 200 ng of genomic DNA, 10 pmol of each primer, and 5 µl of COSMO PCR master mix (Willowfort, Vincent Drive, Birmingham, UK) in a final volume of 20 µl. The PCR amplification was then carried out on Bio-RAD T100 gradient thermal cycler (Bio-RAD, California, USA) with initial denaturation at 95°C for 5 min followed by 30 cycles of denaturation at 95°C for 1 min, annealing at 60°C for 1.5 min, and extension for 1 min at 72°C. A final extension step of 10 min at 72°C was performed at the end. The resultant PCR products were then visualized on 2% agarose gel using 100 bp ladder (Thermo Scientific) as a marker for size confirmation [20].

Identification of the different APOE gene polymorphisms

Restriction fragments length polymorphism was carried out using HhaI restriction enzyme (New England, Biolabs, Ipswich, MA, USA). Five microliters of the amplified PCR products were digested using 1 µl (10 U) of the enzyme at 37°C for 1 h on Bio-RAD T100 gradient thermal cycler (Bio-RAD) as directed by the manufacturer protocol. Digestion products were then visualized on 3% agarose gel to identify the different genotypes of the APOE gene according to the protocol proposed by Hixson and Vernier [20].

Sanger sequencing

The genotypes results were further confirmed by Sanger sequencing. Another 5 µl of the amplified

PCR products were purified using ExoSAP-IT PCR Product Cleanup Reagent (Thermo Scientific) kit and sequenced by the ABI 3500 genetic analyzer (Applied Biosystems, Waltham, MA USA) following the established manufacturer protocols. The obtained sequences were then compared to the human reference genome using the NCBI nucleotide BLAST (Basic Local Alignment Search Tool) available online (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Statistical analysis

Analysis of statistical results was carried out by SPSS 16 (Statistical Package for Scientific Studies, Munich, Germany), GraphPad prism and Microsoft Office Excel 2010. Data of this study were of both quantitative and qualitative types. Parametric data were expressed as means±SD. Nonparametric quantitative data were expressed in median, minimum, and maximum. While qualitative data were expressed in frequency (number) and percent (%). The comparisons between two independent groups with nonparametric comparison of more than two independent groups were done by using χ^2 or Kruskal–Wallis test. Student *t* test was used to compare between two groups with parametric data, and Mann–Whitney test (*U*) to test significance difference between two nonparametric quantitative variables. While analysis of variance test was used to compare between more than two groups. Spearman correlation was used to measure the strength and direction of relationship between two nonparametric variables *P* values less than 0.05 were considered statistically significant.

Results

Sixty participants between the ages of 15 and 60 years were incorporated into this study. There were no statistical differences in sex and age between the epilepsy group and the control group as shown in Table 1.

Clinical evaluation

The onset of epilepsy varied in age from 4 months to 49 years (18.12±10.95 years). No one of the epileptic patients (0%) reported a history of febrile convulsions, none of the patients (0%) had a history of status epilepticus and four patients (10%) had family history of epilepsy. Six (15%) patients experienced daily seizures, three (7.5%) epileptic patients suffered from weekly seizures, 23 (57.5%) patients experienced monthly seizures, and eight (20%) patients were controlled for more than year. Three (7.5%) epileptic patients had morning seizures, three (7.5%) patients had nocturnal seizures, and thirty-four (85%) patients had both. Fourteen (35%) patients received monotherapy, 14 (35%) patients received dual therapy, two (5%) patients obtained triple therapy, and 10 (25%) patients not taken any drug. Interictal EEG results showed that 26 (65%) patients had normal EEGs at the time of recording and 14 (35%) patients had abnormal patterns. Generalized EEG anomalies (spike and sharp waves) constituted the EEG abnormalities as shown in Table 2.

Table 3 shows that the total Addenbrooke score and its items (attention, memory, and fluency) were significantly decreased ($P<0.05$) in patients compared to controls. Meanwhile, there was no significant difference between studied groups regarding language and visuospatial processing ($P=0.161$ and 0.837, respectively).

Molecular results

The Sanger sequencing of the same fragment showing the different genotypes as shown in Figs 1–4. The combination of the two polymorphisms in different phenotypes is presented where figure A always displaying the genotype for the polymorphism rs429358 (c.388 T>C), and figure B presenting the genotype for the other polymorphism rs7412 (c.526 C>T). Figure 1 shows the most common genotype ε3/ε3 found in 34 of the patients and 19 of the controls. Figure 2 shows the second common ε2/ε3 phenotype found in four patients with homozygous rs429358 and heterozygous rs7412. The ε3/ε4 phenotype was found in one patient and one control and is shown in Fig. 3 (heterozygous rs429358 for and homozygous mutant for rs7412). The last phenotype found was ε4/ε4 found

Table 1 Comparison between age and sex in patient and control groups

Variables	Patients (*N*=40)	Controls (*N*=20)	Test of significance	*P* value
Sex [*n* (%)]				
Male	21 (52.5)	8 (40)	χ^2=0.834	0.361*
Female	19 (47.5)	12 (60)		
Age				
Mean±SD	32.2±13.04	36.2±8.78	*U*=297.5	0.108**

*Insignificant difference at *P* value more than 0.05, using χ^2 test. **Insignificant difference at *P* value more than 0.05, using Mann–Whitney (*U*) test.

Table 2 Characteristics of epileptic patients' group

	Patients (*N*=40) [*n* (%)]	
Variable	Positive	Negative
History of epilepsy	4 (10)	36 (90)
Consanguinity	2 (5)	38 (95)
History of status epilepticus	0	40 (100)
History of febrile seizures	0	40 (100)
EEG	14 (35) Showed generalized	26 (65) Normal
Temporal pattern		
Not known	0	
Both	34 (85)	
Nocturnal	3 (7.5)	
Diurnal	3 (7.5)	
Treatment (drug)		
Not taken	10 (25)	
Monotherapy	14 (35)	
Dual therapy	14 (35)	
Triple therapy	2 (5)	
Seizure frequency		
Controlled	8 (20)	
Monthly	23 (57.5)	
Weekly	3 (7.5)	
Daily	6 (15)	

Table 3 The results of the Addenbrooke score evaluation and its items

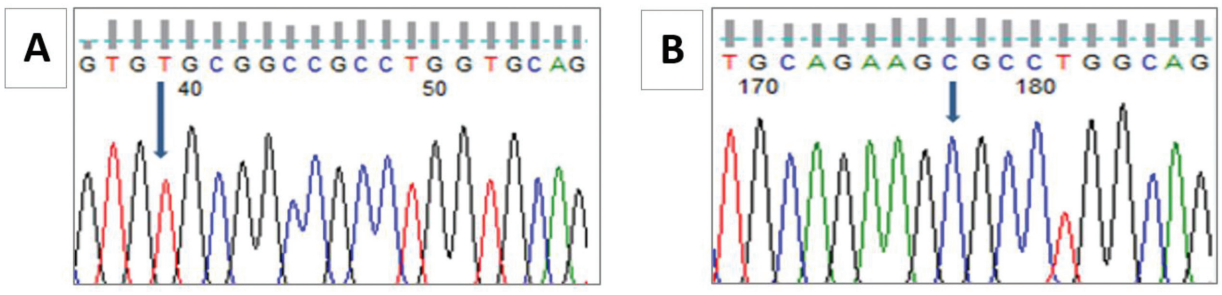
Variables	Patients (*N*=40)	Control (*N*=20)	*P* value
Addenbrooke [*n* (%)]			
Abnormal	39 (97.5)	5 (25)	<0.001*
Normal	1 (2.5)	15 (75)	
Addenbrooke total score (mean±SD)	60.80±12.71	80.60±7.71	<0.001**
Attention (mean±SD)	14.35±2.69	17.6±0.598	<0.001**
Memory (mean±SD)	12.75±4.61	22.45±4.22	<0.001**
Fluency (mean±SD)	1.63±2.86	8.7±2.08	<0.001**
Language (mean±SD)	20.3±3.47	20.1±1.71	0.161
Visuospatial processing (mean±SD)	11.03±3.57	11.55±1.76	0.837

*Significant difference at *P* value less than 0.05, using χ^2 test. **Significant difference at *P* value less than 0.05, using *t* test.

in one patient homozygous for both rs429358 and rs7412 with 2 Arginine residues in 112 and 158 positions, respectively, as shown in Fig. 4.

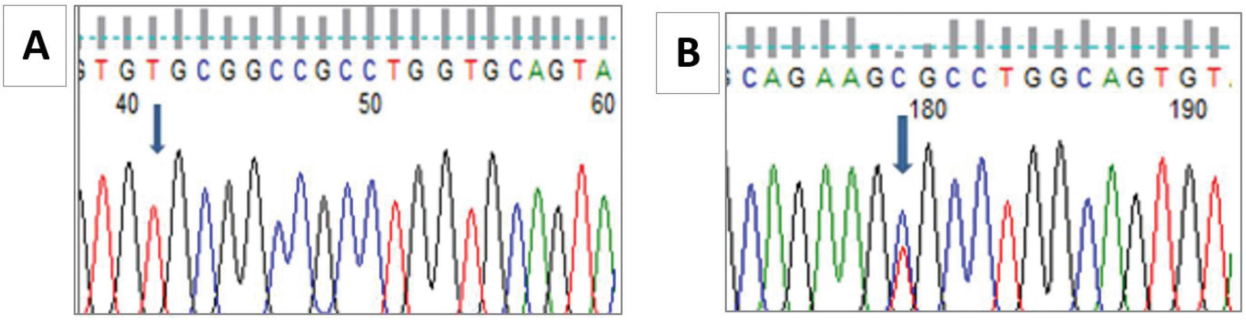
Table 4 summarizes the *APOE* genotypes distribution between both epileptic and control groups. Mutations were found in six patients, four males and two females.

Figure 1



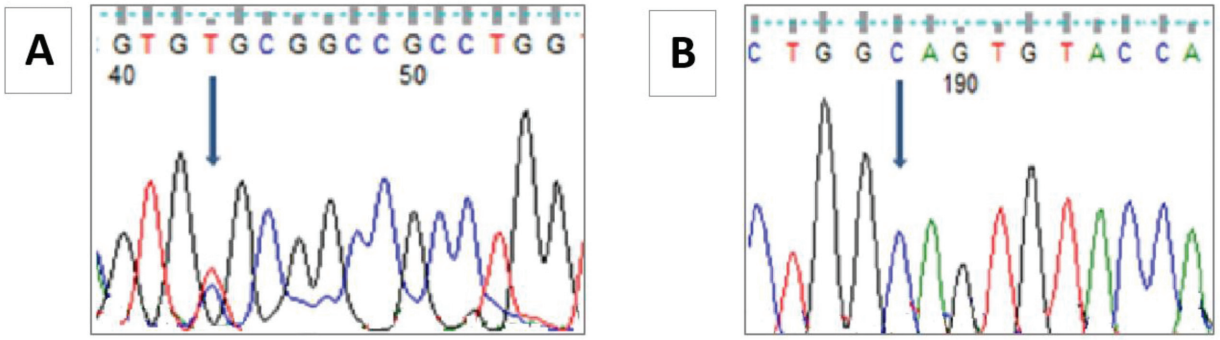
Sequence chromatogram of *APOE* gene representing the genotype ε3/ε3. (a) Represent the homozygous wild genotype of rs429358 (c.388 T>C), (b) represent the homozygous wild genotype of rs7412 (c.526 C>T). Site of genotype determination is denoted by blue arrow.

Figure 2



Sequence chromatogram of *APOE* gene representing the genotype ε2/ε3. (a) Represent the homozygous wild genotype of rs429358 (c.388 T>C), (b) represent the heterozygous genotype of rs7412 (c.526 C>T). Site of genotype determination is denoted by blue arrow.

Figure 3

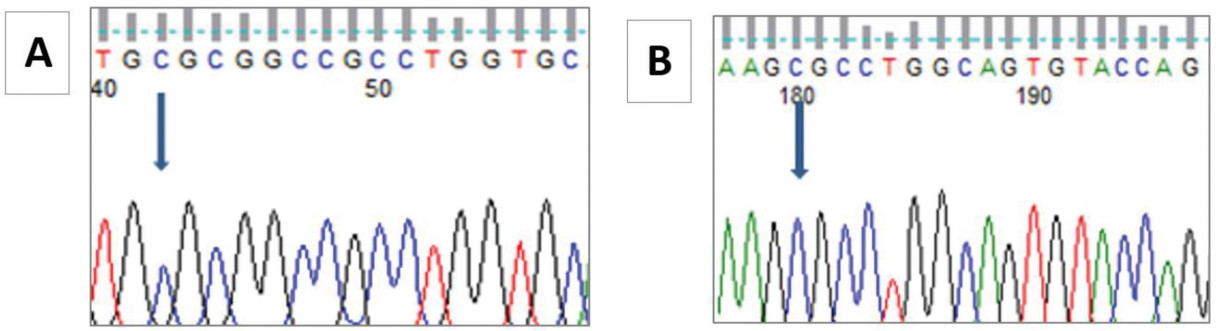


Sequence chromatogram of *APOE* gene representing the genotype ε3/ε4. (a) Represent the heterozygous genotype of rs429358 (c.388 T>C), (b) represent the homozygous wild genotype of rs7412 (c.526 C>T). Site of genotype determination is denoted by blue arrow.

Four patients had ε2/ε3 genotype, while only one patient in each of the following genotypes; ε3/ε4, ε4/ε4. Only one participant of the control group had the genotype ε3/ε4. All other participants had the wild type genotype ε3/ε3; there were insignificant changes in genotype frequencies. The majority of our sample had the wild type genotype ε3/ε3 (85% of patients and 95% of controls).

Based on our findings, no statistically significant differences were found in distribution of Addenbrooke score and parameters according to *APOE* genotype distribution in patients' group, while in controls there was no significant difference in distribution of attention and visuospatial processing parameters according to genotype distribution. Meanwhile, there was significant increase in

Figure 4



Sequence chromatogram of *APOE* gene representing the genotype ε4/ε4. (a) Represent the homozygous mutant genotype of rs429358 (c.388 T>C), (b) represent the homozygous wild genotype of rs7412 (c.526 C>T). Site of genotype determination is denoted by blue arrow.

Table 4 The *APOE* genotypes distribution between both epileptic and control groups

Genotypes	Patients (*N*=40) [*n* (%)]	Control (*N*=20) [*n* (%)]	Test of significance	*P* value
ε3 ε3	34 (85)	19 (95)		
ε2/ε3	4 (10)	–		0.407*
ε3/ε4	1 (2.5)	1 (5)	FEX=2.901	
ε4/ε4	1 (2.5)	–		

*Insignificant difference at *P* value more than 0.05, using Fisher exact test.

Addenbrooke, memory, fluency, and language processing in ε3/ε3 genotype compared to ε3/ε4 genotype (*P*=0.000, 0.007, 0.016, and 0.000, respectively), as shown in Table 5.

The correlation between patients' phenotype and genotypes among the patients' group is presented in Table 6. However, there was insignificant difference in distribution of patient history according to genotype distribution, except with age of onset ($P<0.001$) and age distribution (*P*=0.036). The association of Addenbrooke parameters between both patients and controls group was evaluated in Table 7 using the spearman correlation coefficient. There was highly significant positive correlation between Addenbrooke parameters attention, memory, fluency, language, and visuospatial processing ($P<0.001$ for all) among the patients' group. While for the control group, there was positive significant strong correlation was found between Addenbrooke parameters attention, memory, and fluency only (*P*=0.013, 0.001, and 0.003, respectively).

Discussion

Around the world, about 50 million people have epilepsy, a brain disease with one of the highest occurrence rates among central nervous system diseases [2]. Individuals affected with IGE has aberrant electrical activity occurring simultaneously from both sides of the cerebral hemispheres. Yet, there is no evidence of structural brain abnormalities on their MRI results [8]. Poorly controlled epileptic seizures have devastating impacts on both psychological and physical health of affected individuals [4]. Lack of intellectual disability and/or

Table 5 Distribution of Addenbrooke cognitive examination test parameters according to *APOE* genotypes in epileptic patient and control groups

	Patients (*N*=40)					Controls (*N*=20)		
	ε3/ε3 (*N*=34)	ε2/ε3 (*N*=4)	ε3/ε4 (*N*=1)	ε4/ε4 (*N*=1)	*P* value*	ε3/ε3 (*N*=19)	ε3/ε4 (*N*=1)	*P* value
Addenbrooke percentage								
Mean±SD	60.85±13.24	64.5±8.39	60±0.0	45±0.0	0.653	81.84±5.49	57.0±0.0	0.000**
Median (minimum–maximum)	63.0 (31–86)	61.0 (59–77)				84.0 (66–87)		
Attention								
Mean±SD	14.3±2.7	16.3±1.71	14±0.0	10±0.0	0.215	17.63±0.60	17.0±0.0	0.316
Median (minimum–maximum)	14.0 (8–18)	16.5 (14–18)				18.0 (16–18)		
Memory								
Mean±SD	12.6±4.9	15.3±1.5	12±0.0	10±0.0	0.600	23.0±3.53	12.0±0.0	0.007**
Median (minimum–maximum)	13.0 (5–26)	15.0 (14–17)				24.0 (15–26)		
Fluency								
Mean±SD	1.65±2.97	2.25±2.63	2±0.0	1.8±0.0	0.470	8.95±1.81	4.0±0.0	0.016**
Median (minimum–maximum)	0.50 (0–14)	1.5 (0–6)				10.0 (3–10)		
Language								
Mean±SD	20.1±3.7	22.0±0.82	23±0.0	18±0.0	0.698	20.42±0.96	14.0±0.0	0.000**
Median (minimum–maximum)	21.5 (14–25)	22 (21–23)				20.0 (19–23)		
Visuospatial processing								
Mean±SD	11.1±3.65	11.5±3.7	11±0.0	7.0±0.0	0.674	11.63±1.77	10.0±0.0	0.381
Median (minimum–maximum)	11.5 (2–16)	12.0 (7–15)				12.0 (9–15)		

*Insignificant difference between Addenbrooke parameters in patients' group at *P* value less than 0.05, using Kruskal–Wallis test.
**Significant difference between Addenbrooke parameters in controls' group at *P* value less than 0.05, using independent *t* test.

Table 6 Distribution of patient's phenotypic parameters according to *APOE* genotypes among epileptic group

Variables	ε3/ε3 (*N*=34) [*n* (%)]	ε2/ε3 (*N*=4) [*n* (%)]	ε3/ε4 (*N*=1) [*n* (%)]	ε4/ε4 (*N*=1) [*n* (%)]	*P* value
Sex and age					
Sex					
Male	17 (50)	4 (100)	0	0	0.104
Female	17 (50)	0	1 (100)	1 (100)	
Age (years)					
Mean±SD	31.18±12.04	47.8±12.5	17±0.00	20±0.00	0.036*
Family history					
History of epilepsy					
Yes	30 (88.2)	4 (100)	1 (100)	1 (100)	0.998
No	4 (11.8)	0	0	0	
Consanguinity					
Yes	33 (97.1)	3 (75)	1 (100)	1 (100)	0.288
No	1 (2.9)	1 (25)	0	0	
Clinical data					
Age of onset (years)					
Mean±SD	16.42±8.57	37.25±11.24	17.00±00	0.42±0.00	0.000*
Seizure frequency					
Controlled	8 (23.5)	0	0	0	0.285
Monthly	20 (58.8)	2 (50)	0	1 (100)	
Weekly	2 (5.9)	1 (25)	0	0	
Daily	4 (11.8)	1 (25)	1 (100)	0	
Temporal pattern					
Both	30 (88.2)	3 (75)	0	1 (100)	0.085
Nocturnal	1 (2.9)	1 (25)	1 (100)	0	
Diurnal	3 (8.8)	0	0	0	
EEG (at the time of recording)					
Showed generalized	12 (35.3)	2 (50)	0	0	0.844
Normal	22 (64.7)	2 (50)	1 (100)	1 (100)	
Treatment					
Drug status					
Not taken	7 (20.6)	2 (50)	1 (100)	0	
Monotherapy	13 (38.2)	1 (25)	0	0	0.646
Dual therapy	12 (35.3)	1 (25)	0	1 (100)	
Triple therapy	2 (5.9)	0	0	0	
Drug-adherence (*N*=30)					
High adherence	14 (51.9)	2 (100)	0	0	
Medium adherence	7 (25.9)	0	0	1 (100)	0.737
Low adherence	6 (22.2)	0	0	0	

All data are represented as numbers (percentage) except for age and sex (represented as mean±SD). *Significant difference at *P* value less than 0.05, using analysis of variance test.

Table 7 Spearman correlation between Addenbrooke parameters in epileptic patient and control groups

	Patients (*N*=40)		Control group (*N*=20)	
	r	*P* value	*r*	*P* value
Age (years)	−0.026	0.872	−0.012	0.959
Attention	0.568	0.000*	0.543	0.013**
Memory	0.856	0.000*	0.760	0.001**
Fluency	0.437	0.000*	0.630	0.003**
Language	0.715	0.000*	0.118	0.621
Visuospatial processing	0.560	0.000*	0.112	0.638

*Significant positive correlation at *P* value less than 0.05, among patients' group. **Significant positive correlation at *P* value less than 0.05, among controls' group.

specific neurological deficits are characteristics of IGEs, but they are characterized with cognitive dysfunction. IGE's source of cognitive dysfunction is still unknown [21].

In addition to maintaining the environmental stability of cholesterol and the redistribution of internal lipids, *APOE* involved in the metabolism of cephalin. It also has a role in the nervous system development, lipid immunoregulation, cellular control, and lipid activities during the regeneration and repair of damaged nerve cells [12]. *APOE* gene can be found in three different genotypes as a result of two naturally occurring SNPs. The three alleles namely are ε2, ε3, and ε4 [22].

A study carried out on Egyptian Alzheimer patient highlighted a significant association between *APOE* ε4 isomer and Alzheimer's dementia among Egyptian patients, and this explained by recent reviews which concluded that *APOE* ε4 raises the pro-inflammatory response, which damages the blood–brain barrier and results in cognitive impairments [23]. The *APOE* ε4 carriers have increased excitability of the network because of the decrease in GABAergic interneuron function [24,25]. In patients with Alzheimer's disease, *APOE* ε4 gene carriers accounted for 30–50% of the total, but in normal populations, the rate was between 14 and 16%. The *APOE* ε4 allele is associated with increased brain amyloid protein deposition and an increased risk of illness onset [12].

Here we evaluated the relationship between specific *APOE* genotypes or alleles and the risk of epilepsy on the one hand, and cognitive function on the other. This study revealed that ε3 allele which carries the ε3/ε3 genotype was commonest in the patients as well as the controls and there was no significant difference in prevalence of ε3 in both groups. This finding is consistent with the earlier international reports and also with studies conducted in normal populations earlier in 2016 and 2021. The results of both investigations indicated that the most prevalent genotype in both groups was *APOE* ε3/ε3 (adult and children) [26,27].

Also, there were no statistically significant differences in genotype between both epileptic and control groups, the ε3/ε3 genotype was the most common genotype found (85% of cases and 95% of controls). This indicates that ε3/ε3 genotype has no correlation for epilepsy, this confronts what was reported in 2006 by Kumar *et al.* [28], whose study showed that a neuroprotective function for the ε3/ε3 genotype in injury and repair was discovered. The authors referred this role to the ability of *APOE* to enhance glutamate uptake and prevent excitotoxicity. The same results were also confirmed by another study in 2019 [29]. The study showed that the risk of epilepsy was higher in ε4 carriers as compared to ε3/ε3 genotype. It concluded that the neuroprotective role of *APOE* was referred to its ability to augment the role of glutamate uptake. This disagreement might be due to ethnic difference, where the ε3/ε3 genotype is more common in population than ε4 and so, its percentage would be higher in the study, which in turn needs further studies to confirm the results [29].

For the total Addenbrooke score, we found a significant decrease in scores in patients' group compared to controls ($P<0.00$) regardless the genotype, the group with epilepsy observed sever cognitive dysfunction (97.4%) below 83 according to Addenbrooke test score, while for controls it is only (22.2%), and this indicates that epilepsy may be the cause for that low score. This was in line with other studies that detected cognitive dysfunction in epileptic patient as in Holmes, who said that typical comorbidities of epilepsy include cognitive dysfunction, including memory and attention problems, and patients with epilepsy often experience a high proportion of cognitive problems that hinder their ability to succeed and educational progress throughout life. A correlation between IGE and cognitive dysfunction was also evidenced in both human and animal models, where seizures regardless of their origin degrade cognitive functioning [5].

As per our study, memory, attention, and language deficits are some of the most reported cognitive complaints in adult patients with epilepsy. In 2020, IGEs were found to be characterized by the absence of intellectual disability and/or focal neurological deficits. Potentially, the continuous epileptogenic processes cause irreversible brain damage, leading to global intellectual deficiencies and permanent cognitive impairment [5,21,30].

There was no significant difference in distribution of Addenbrooke and its parameters and genotype in epileptic patients, and this is not goes in hand with other study that indicate that the lifetime risk of moderate cognitive impairment is 30–35% for *APOE* ε4 homozygote individuals, 20–25% for *APOE* ε4 heterozygote adults (ε3/ε4 and ε2/ε4), and 10–15% for non-*APOE* ε4 individuals (ε3/ε3, ε3/ε2, and ε2/ε2), according to a population-based cohort research [30,31]. *APOE* ε4 was also reported to affect cognition even in heterozygous pattern (ε2/ε4 and ε3/ε4) compared to homozygous ε3 alleles genotype [19]. This is ought to its neuroprotective role in injury and repair, where it controls calcium homeostasis, protects neurons from oxidative stress after damage, and modifies both innate and adaptive immunological responses [30].

Regarding the correlation between the age of onset and *APOE* genotype, we noticed the onset was early adulthood in case of ε3/ε4 and ε4/ε4 genotypes (17 and 20 years, respectively), and was delayed to elder age in case of ε2/ε3 and ε3/ε3 and ε2/ε3 genotypes. This is consistent with a 2021 study found that the average age of disease onset for patients carrying the *APOE* ε2/ε3 genotype was 10 years greater than that of patients

carrying the *APOE* ε3/ε4 genotype, and 6 years greater than that of patients carrying the *APOE* ε3/ε3 genotype [29].

Conclusion

Based on our findings of this study, since *APOE* ε3 is a common genotype in both patient and control group, the effect of *APOE* genotype on idiopathic epilepsy incidence is minimal. On the other hand, a positive association exists between presence of idiopathic epilepsy and the decline of cognitive functions based on the Addenbrooke score parameters. Therefore, patients diagnosed with IGE require special care to enhance their defective cognition. Yet, large-scale study is needed to confirm the correlation between *APOE* genotype and IGE to assess positive or negative results.

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Conflicts of interest

There are no conflicts of interest.

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