Association of GRIN2A (rs387906637) Gene Polymorphism with Epilepsy Susceptibility

Akram Jawad Hameedi, Asmaa Mohammed Saud

Biotechnology Department, College of Science, University of Baghdad ,Baghdad, Iraq

Abstract

The aim of this study was to investigate the correlation between GRIN2A rs387906637 polymorphism and susceptibility to epilepsy. Blood samples were collected from 85 volunteers, dividing into 60 epilepsy patients (34 males and 26 females) and 25 healthy subjects (19 males and 6 females).The DNA was extracted and GRIN2A rs387906637 polymorphism was analyzed by Real-time PCR using two probes and primers. The results showed no significant differences between patients and control samples; therefore, there are no allelic and genotypic correlations of this SNP with epilepsy. This study indicated that GRIN2A rs387906637 polymorphism is not a risk factor for epilepsy in the studied set of patients.

Keywords: Epilepsy, GRIN2A, (rs387906637) Polymorphism, Real-time PCR.

ارتباط تعدد الأشكال لجين GRIN2A (rs387906637) مع قابلية التأثر بالصرع

اكرم جواد حميدي*, اسماء محمد سعود

قسم التقنيات الإحيائية, كلية العلوم, جامعة بغداد, بغداد, العراق

الخلاصة

تهدف هذه الدراسة إلى التحقق من العلاقة بين تعدد الإشكال لجين GRIN2A rs387906637 مع قابلية التأثر بالصرع . عينات الدم تم جمعها من 85 متطوع (60 عينة أخذت من مرضى الصرع و25 عينة تم أخذها من ناس سليمين) متكونين من 34 ذكر و 26 أنثى مصابين بالصرع و19 ذكر و6 إناث سليمين من أي مرض. DNA تم استخلاصه وتعدد الإشكال لجين GRIN2A rs387906637 تم فحصه بواسطة RT−PCR باستخدام برايمرين وبروبين . أظهرت النتائج بأنه لا يوجد اختلاف معنوي بين rs387906637 ومرض الصرع عند مقارنة عينات المرضى مع عينات الناس السليمين. أشارت هذه الدراسة إلى أن تعدد الأشكال GRIN2A rs387906637 ليس عامل خطر للصرع في مجموعة المرضى المدروسة.

1. Introduction

Epilepsy is a central nervous system condition that results from brain disorder and characterized by chronic predisposition to form seizures. The disease requires two unprovoked seizures in addition to secondary neurobiological, psychological, cognitive, and social consequences. The period between the first and second seizures is typically one day [1]. Epilepsies that are caused by genetic, developmental, or congenital conditions are more prevalent among young people, while brain tumors and strokes often affect the elderly [2]. Seizures can also occur as a result of other health problems [2]; these are known as acute symptomatic seizures when they occur due to specific causes, such as toxic ingestion, stroke,

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*Email: akrmiq506@yahoo.com

head injury, or metabolic problems, and more generally classified as seizure-related disorders than as epilepsy itself [3–4]. Genetics is believed to be directly or indirectly involved in most cases [5]. Certain cases of epilepsy are caused by a single gene defect; it should be noted that this monogenic epilepsy accounts for only a small minority (1–2 %) of all human epilepsy cases. Most cases of epilepsy are multifactorial in origin, caused by the interaction of multiple genes, and possibly further influenced by environmental factors [5]. The majority of epilepsy cases result from an acquired "symptomatic" cause; for example, moderate brain injury increases the risk by about two-folds, while the risk rises by seven-folds in severe brain injuries [6]. The risk is about 50% in those who have suffered a high-powered gunshot wound in the head [7]. Epilepsy risk following viral or bacterial meningitis infection is less than 10%; this illness causes seizures more frequently during the infection itself [8]. However, the disease is familiar in Iraq and most epilepsy cases in Baghdad and Erbil occur in the first and second decades of life in both genders, although the age has no significant difference [9–10]. Recently, molecular analysis tests have been widely used in Iraq to detect and diagnose genetic disorders such as epilepsy, schizophrenia and asthma; for example, genotyping was used to explain the relationship between 5-HTR2A gene and schizophrenia [11]. Also, diabetes was studied in Iraq by detecting the correlation between IL-10 (-592A/C) and IL-2 gene polymorphism with progression of Type 2 Diabetes Mellitus [12–13]. These two examples explain the importance of molecular tests in the analysis of genetic disease. For this reason, genotyping was used in this study to explain the role of rs387906637 in epilepsy.

Defect in the NMDA subunit 2A (GRIN2A) ionotropic glutamate receptor has been associated with pharmacoresistant epilepsy. This type of epilepsy, also called refractory epilepsy, is not affected by anti-epileptic drugs [14]. N-methyl-D-aspartate (NMDA) is a class of receptors called ionotropic glutamate receptors. Mutations in GRIN2A gene are associated with a number of neurological disorders, such as focal epilepsy and speech disorder [15]. There is a long-established correlation of NMDAR dysfunctions with multiple neurodevelopmental disorders [16–17]. Many researchers identified a large number of heterozygous mutations in GRIN2A gene that encodes the GluN2A subunit of NMDARs, in people with epilepsy-aphasia spectrum disease [18–19–20]. GRIN2A disruption in translocation subjects and nonsensical modification would likely lead to functional null alleles correlated with a relatively mild phenotype consisting of epilepsy and different cognitive impairments [21].

However, epilepsy inheritance is complex and difficult to interpret ate; for example, it occasionally occurs that two children with defect or mutation in different genes may develop the same epilepsy syndrome [22]. In other cases, two children of the same family with a defect or mutation in the same gene may develop epilepsy with different effects. Some epilepsy syndrome, even though it is known that they have a genetic basis, the exact causative genes are not known [22]. GRIN2A gene has rs387906637 that is considered as a candidate SNP that may cause epilepsy. rs387906637 is present in exon 4 at the allele "C" which is found in the codon that encodes the amino acid Glutamine (Gln) [23]. This SNP in the location 249441C >T converts to a stop codon in the case of mutation, where this change leads to replace Gln218 to the termination codon, causing the occurrence of stop-gain mutation and, therefore, termination of translation [23]. In the research field, there is generally a little attention to GRIN2A gene or its SNP, with no related previous studies in the Iraqi population. Thus, we attempt in this study to focus on the genetic polymorphism which could be related to epilepsy. In order to achieve that, the genetic polymorphism of rs387906637 from the study groups was genotyped by real-time PCR technique.

2. Materials and Methods
Blood Collection
Blood samples (5 ml) were taken from eighty-five volunteers and divided into two groups; the first group included 60 samples taken from epilepsy patients (34 males and 26 females) with age range of 26-49 years, while the second group included 25 samples taken from healthy people (19 males and 6 females) with age range of 25-53 years. Sample collection period extended from November 2018 to April 2019. Patients' blood samples were collected from the Neurosciences Hospital, Baghdad, Iraq. Diagnosis was performed according to the ILAE (International League Against Epilepsy) and confirmed by psychiatric observation, information about clinical ground, and electroencephalogram (EEG) findings [24]. All participants were of unrelated Iraqi origin and had similar geographic and

socio-demographic data. The patients provided their informed written approval to participate in the study and allowed the usage of their samples for DNA isolation .

DNA Extraction

Genomic DNA was isolated from blood samples in compliance with the protocol of ReliaPrep™ Blood gDNA Miniprep System kit (promega / USA). Polymorphism rs387906637 was assessed by real time polymerase chain reaction (Real-time PCR) and Sanger sequencing. All primers and probes were newly designed in this study and ordered from Macrogene Company (Korea).

Genotyping

The genetic polymorphism of rs387906637 within GRIN2A gene in the location 249441C >T was the selected variant to be genotyped by Real-time PCR technique. The Real-time PCR was performed with the forward primer GRIN2A : 5`-CTGGACACTTCCTTTGAG-3` and reverse primer GRIN2A : 5`-AGAGCAAGATGACAGAAG-3`. In addition, two probes were used, namely the probe allele C: Fam-5`-AAGACACAAGTCCAGCTGAAGAAGATC-3` to detect C allele and the probe allele T : Hex-5`-AAGACACAAGTCTAGCTGAAGAAGATC-3` to detect T allele .

The reaction was performed in a Mic qPCR Cycler from Bio Molecular System, Australia, using GoTag qPCR Master Mix kit. The 10µl PCR solution contained 5µl of GoTag qPCR 2x Master Mix (Promega, USA), 0.5µl of each 10µM primer (forward and reverse primers), 0.5µl of each 10µM probe (Probe allele C and Probe allele T), 2 of µl nuclease-free water, and 1µl of DNA. The reaction was performed according to the following steps: initial denaturation at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds .

Standard Sequencing

Random samples were rechecked by Sanger sequencing. The PCR process was performed using the forward primer GRIN2A : 5`-GACGCAGTTTGTGCTTCTA-3` and reverse primer GRI2NA: 5`-CAATCCCAGGTGTAGGTTTC-3` .

PCR products were sent for Sanger sequencing using ABI3730XL, automated DNA sequencer, by Macrogen Corporation – Korea.

Statistical analysis

Data analysis was performed by utilizing SPSS for Windows, version 22 (SPSS Inc. Chicago, Illinois, United States). Shapiro–Wilk normality test was used to determine whether the studied parameters followed a Gaussian distribution. Data are displayed as mean ± standard deviation (SD). Student's t-test was used to compare between means of the studied groups. Additionally, categorical variables were analyzed by Chi-square test. Hardy-Weinberg equilibrium (HWE) was calculated using a web tool [25]. Odds ratios (ORs) with a 95% confidence interval (CI) were also calculated. A two-tailed p value ($p < 0.05$) was considered significant [26].

3. Results

Standard deviation (SD) for age was calculated for both control (39.04±13.33) and patients (39.69±10.02) groups. Most of epilepsy cases in this study had an age of less than 39 years, but the results showed that epilepsy emerges at an age average of 9.32±4.58 (Table-1).

Table 1-General characteristics of the studied groups

Characteristics	Control group (N=25)	Patients group (N=60)	*P* value
Age	39.04±13.33	39.69±10.02	0.80
Gender Male Female	19 (76.0 %) 6 (24.0 %)	34 (56.7 %) 26 (43.3 %)	0.10
Family history NO Yes		34 (56.7%) 26 (43.3%)	
The age of epilepsy onset	__	9.32±4.58	__

Data were expressed as counts, with percentages in parentheses, or as mean ± SD.

The results showed that the age and gender had no significant differences ($p = 0.80$ and 0.10, respectively). Our study also showed that the number of epilepsy patients with family history was 26 (43.3%), whereas 34 (56.7%) patients had no family history.

Genotyping and allele frequencies of GRIN2A

Genotyping of rs387906637 by Real Time PCR showed no variants in this region, where all the 85 samples of patients and controls contained the allele CC (Table-2).

Table 2-Frequencies of GRIN2A gene genotypes and their Hardy-Weinberg equilibrium (HWE) in the control and patient groups

Genotype	Control group (n=25)		*p* value	patients group (n=60)		*p* value
	Observed n (%)	Expected n (%)		Observed n (%)	Expected n (%)	
CC	25 (100)	25 (100)	*P*>0.05	60 (100)	60 (100)	*P*>0.05
CT	0 (0)	0 (0)		0 (0)	0 (0)	
TT	0 (0)	0 (0)		0 (0)	0 (0)	

1 degree of freedom (d.f.) for Chi-squared distribution.

Therefore, no significant difference was observed in allele frequency and genotyping of rs387906637 polymorphism in epilepsy patients when compared to healthy control volunteers (Table-3).

Table 3-Genotype and allele frequencies of GRIN2A gene in control and patients groups

Genotype / Allele	Control group (n=25)	Patients group (n=60)	OR	95% CI	*p* value
	no. (%)	no. (%)			
CC	25 (100)	60 (100)	0.42	0.008-21.82	0.66
CT	0 (0)	0 (0)	2.37	0.04-122.87	0.66
TT	0 (0)	0 (0)	2.37	0.04-122.87	0.66
C	50 (100)	120 (100)	0.41	0.008-21.41	0.66
T	0 (0)	0 (0%)	2.39	0.04-121.92	0.66

OR, odd ratio; CI, confidence interval.

The standard sequencing is the optimal method to detect DNA sequence. However, random samples were rechecked by Sanger sequencing to ensure the accuracy and to make verification if there are any mistakes in the detection of rs387906637 by Real-time PCR Figures-(1 and 2).
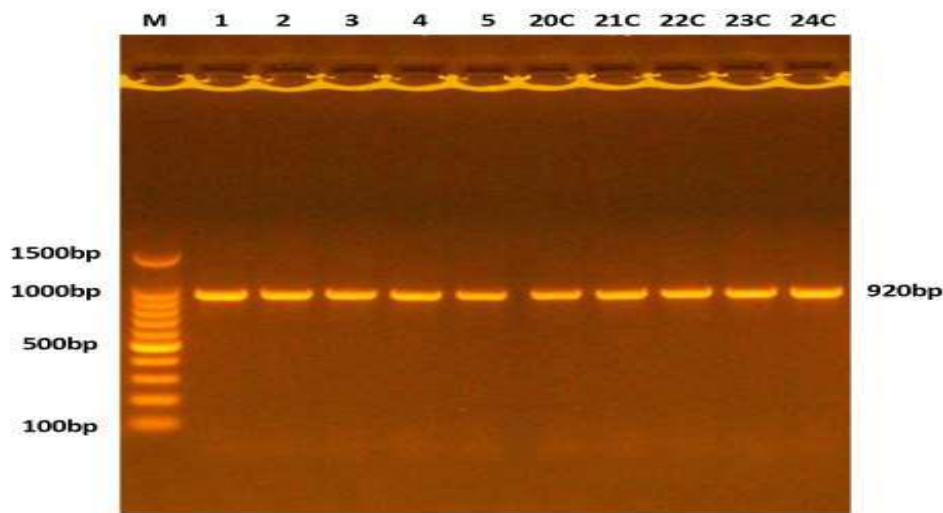
Figure 1-Gel electrophoresis of selected regions for GRIN2A gene, fractionated on 1% agarose and stained with Eth.Br. Lane1:100bp DNA marker; lanes 2-11: DNA samples.
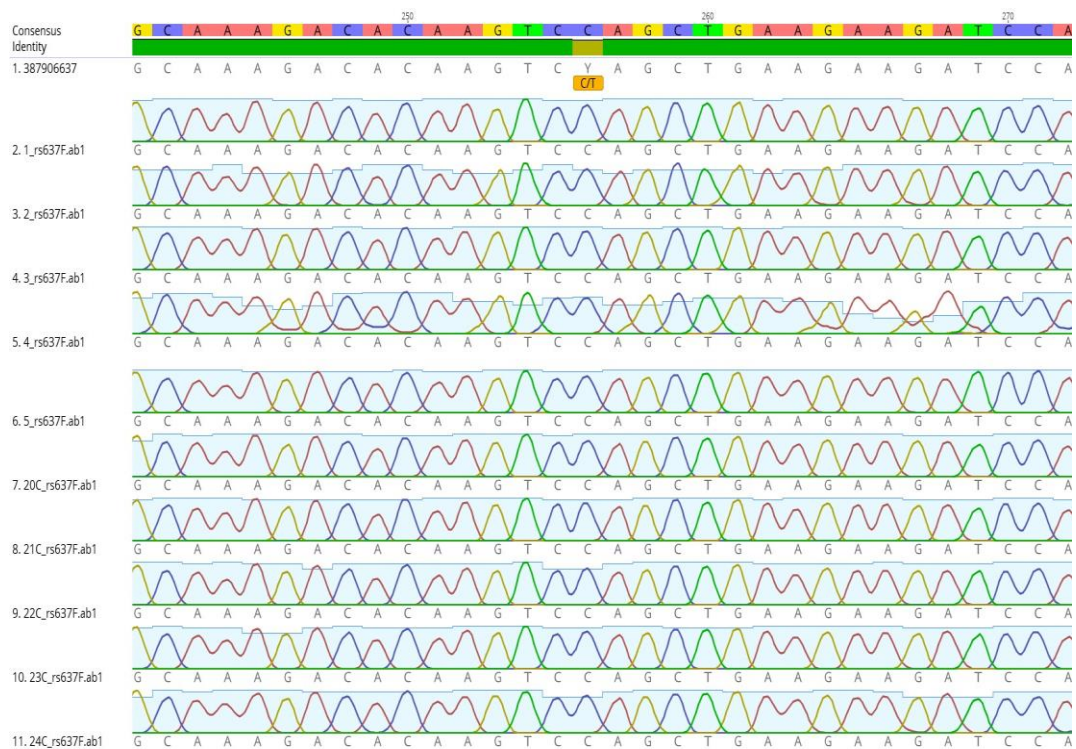

Figure 2-Nucleotides sequence at rs387906637 region detected by Sanger sequencing, which confirmed that C allele is present in all samples.

4. Discussion

In this study, genotyping of GRIN2A was performed at rs387906637 by RT-qPCR for 85 volunteers (60 epilepsy patients and 25 healthy controls) in Baghdad, Iraq. The results showed no significant differences between the genotypes in the patients and controls, where all the samples contained the same genotype (CC). Several random samples were re-tested by direct sequencing to ensure accuracy, however, similar results were found. According to the National Center for Biotechnology Information (NCBI), the mutation in rs387906637 causes a stop-gain mutation and leads to the termination of protein translation [23]. However, our study did not record any variant at the SNP region in both patients and healthy subjects, indicating no relationship between rs387906637 and epilepsy in this sample of Iraqi population.

From the analysis of genotyping and allele frequency for the sixty epilepsy patients who participated in this study , CC genotype was identified in 60 (100%) samples. CC ratio in the twenty five controls was 25 (100%). Whereas CT and TT genotypes were not identified in any sample of both

controls and epilepsy patients . The odds ratio (OR) values with 95% confidence intervals (CI) and p value were also calculated. Subjects who have the CC genotype are more susceptible to epilepsy, as indicated by OR of 0.42, 95%CI of 0.008-21.82, and p v value of 0.66.

Analysis of allele distribution showed that the frequency of C allele was 120 (100%) in epilepsy patients and 50 (100%) in healthy controls. Epilepsy patients with C allele frequency were found to have a 0.41 fold higher risk of developing the disease as compared to the control group (OR, 0.41; 95% CI, 0.008-21.41; P=0.66) (). While the frequency of T allele was 0% in both patients and control groups (Table-3). For this reason, no significant differences were identified in allele frequency and genotyping of rs387906637 polymorphism in patients with epilepsy when compared to healthy control.

Mutations in GRIN2A have been associated with epilepsy-aphasia spectrum disorders. According to Carvill *et al* (2013), more than 30 GRIN2A missense mutations were found in patients with these disorders [27, 28]. The relationship between GRIN2A mutations and epilepsy-aphasia spectrum has gained remarkable interest [29–30]. It has been proposed that destructive mutations in GRIN2A can lead to the change of the non-expressed GluN2A subunit along with other functionally distinct subunits [31]. However, recently published findings do not appear to support this hypothesis [32].

The present results found no significant differences between age/gender and epilepsy, which implies that a person can have epilepsy at any age in both males and females. This study comes in accordance with other studies in Baghdad and Erbil cities (in Iraq) that showed no significant differences between epilepsy and age or gender [9, 10]. Epilepsy mostly develops in children and adolescents, but it can start at any age [33]. In the current study, the mean value for age of epilepsy onset is 9.32±4.58 years. According to the results, the minimum age of epilepsy onset is 5 years (9 .32-4.58≈ 5) and the maximum value is 13 years (9 .32+4.58≈ 13) (Table 1). In Baghdad, most epilepsy cases occurred during childhood at age less than nine years. This can be due to the fact that many children in Iraq might be born with a genetic defect and they have more probability to be influenced by environmental factors, such as vehicle accident, head trauma, brain tumor, and infection diseases, in addition to the effects of wars. Despite that epilepsy can emerge at any age, many studies stated that epilepsy has a high ratio at specific ages. Two studies from Tunisia and Saudi Arabia showed that, in most cases, the disease prevalence in young adults and children is higher than people in middle age by two folds [33-35]. These studies also found that disease prevalence becomes higher in elderly who have age over 60 years [34, 35]. Several researchers attempted to understand the relationship between gender and epilepsy, and their studies showed that males have a higher rate of disease than females [36, 37]. Nevertheless, it is challenging to consider this ratio as statistically relevant [38], because in many other studies, different findings showed that the number of males with epilepsy is less than that of females [39].

In the present study, the number of patients who have family history with epilepsy is 26 (43.3%), whereas 34 (56.7%) have no family history. This might be attributed to an idiopathic cause of epilepsy. Hauser *et al.* (1990) and Jallon *et al.* (2001) demonstrated that genetic factors have a major role to play in causing epilepsy in 30% of all cases [38–40]. If epilepsy is developed as a consequence of a genetic cause, it is considered as idiopathic epilepsy [38–40]. While symptomatic epilepsy is the consequence of acquired conditions, such as head trauma, infectious diseases, tumor, and several other neurological disorders. However, epilepsy can be caused by a combination of acquired and genetic factors [41–42]. demonstrated Accordingly, our results suggest that 56.7% of patients have acquired or "symptomatic" epilepsy. This result is consistent to those of Jallon *et al.* (2001) and Hauser *et al.* (1990), who found that symptomatic epilepsy accounts for 60% of all epilepsy cases [38–40].

5. Conclusions

Genotyping of GRIN2A showed a lack of correlation between rs387906637 and epilepsy in the studied Iraqi epileptic cases. The study also showed no significant differences between age/gender and epilepsy, while family history ratio promotes the probability of a genetic origin for some epileptic cases.

Conflict of Interest The authors declare that they have no conflict of Interest.

5. Acknowledgments

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