Effects of GRM4, SCN2A and SCN3B polymorphisms on antiepileptic drugs responsiveness and epilepsy susceptibility

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ABSTRACT

Background: Pharmacotherapy of epilepsy including antiepileptic drugs (AEDs) is one of the main treatment approaches. As a biological target, sodium channels (Nav channels) and glutamate receptor genes are playing a major role in the etiology and treatment of epilepsy.

Objective: This study aims to investigate the genetic associations of certain genetic polymorphisms with increased risk of epilepsy susceptibility and variability in response to AEDs treatment in a Jordanian Arab population.

Method: A pharmacogenetics and case-control study on 296 unrelated epileptic Jordanian patients recruited from the pediatric neurology clinic at the Queen Rania Al-Abdullah Hospital (QRAH) in Amman, Jordan and 299 healthy individuals was conducted. Children up to 15 years old which receiving AEDs for at least three months were scanned for genetic association of 7 single nucleotide polymorphisms (SNPs) within three candidate genes (SCN2A, SCN3B and GRM4) with epilepsy susceptibility.

Results: SCN2A rs2304016 ($P = 0.04$) and GRM4 rs2499697 ($P = 0.031$) were statistically significant with generalized epilepsy. Haplotype of CAACG GRM4 was genetically associated with epilepsy and partial epilepsy ($P = 0.036$; $P = 0.024$, respectively). This study also found that TGTAA genetic haplotype formed within GRM4 gene was associated with generalized epilepsy susceptibility ($P = 0.006$). While, no significant linkage of SCN3B rs3851100 to either disease susceptibility or drug responsiveness was found.

Conclusion: This study identified no significant associations of allelic or genotypic SNPs with the susceptibility of epilepsy and medication response with an exception of rs2304016 and rs2499697 SNPs that were associated with the generalized type of epilepsy among Jordanian population. Further studies are required in different populations to confirm our results and identify genetic factors that involved in susceptibility and treatment response.

1. Introduction

Epilepsy is a brain disorder identified by an enduring predisposition to generate epileptic seizures with occurrence of one seizure at least (Fisher et al., 2014). Epilepsy is often associated with obvious causes including neurodevelopmental abnormalities, central nervous system (CNS) trauma and inflammation (Shneker and Fountain, 2003; Beck and Elger, 2008). It is believed that epilepsy disorder could have a certain genetic contribution with polygenic and multifactorial inheritance (Sisodiya and Duncan, 2004; Steinlein, 2008). Currently, there are more than 15 available antiepileptic drugs (AEDs) with different chemical structure and mode of action used for epilepsy disorder treatment (Schachter, 2007; Depondt and Shorvon, 2006). AEDs are primarily function to increase inhibition, decrease excitation, and/or prevent aberrant burst firing of neurons to prevent epileptic seizures (Mann and Pons, 2007; Haerian et al., 2013). Drugs must act on one or more targets in the brain such as ion channels, neurotransmitter transporters and neurotransmitter metabolic enzymes in order to exhibit the antiepileptic activity (Kwan et al., 2001). They could act by modulation of voltage-dependent ion channels, enhancement of gamma-aminobutyric acid (GABA)-mediated inhibitory neuro-

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transmission, and attenuation of excitatory glutamate-mediated transmission (Schachter, 2007; Meldrum and Rogawski, 2007). Ion channel genes (e.g. sodium voltage-gated channel alpha subunit 2 (*SCN2A*) and beta subunit 3 (*SCN3B*)) are considered as a major target for many ADEs (Haerian et al., 2013). Experimental molecular biological studies of have shown that channel affinity for the drug can be improved or reduced by genetic mutations in ion channel genes. On the other hand, these genes have been reported to be associated with various epilepsy phenotypes (Lakhan et al., 2009; Haerian et al., 2013). Furthermore, *GRM4* gene encodes the metabotropic glutamate receptor 4 (mGluR4) which have multiple actions on neuronal excitability through G-protein-linked receptors (GPCRs) modifications of enzymes and ion channels (Parihar et al., 2014; Moldrich et al., 2003). Excessive glutamatergic neurotransmission is one of the primary mechanisms behind the etiology of epilepsy subtypes. For example, earlier studies in animals showed that glutamate was capable of inducing epilepsy (Chapman et al., 1996; Meldrum, 1991). Moreover, glutamate receptors (*mGluR*) genes are one of the potential targets for AEDs in the treatment of epilepsy (Moldrich et al, 2003). Thus, we hypothesized that genetic variation within *SCN2A, SCN3B* and *GRM4* genes may influence both, the responsiveness to AEDs (e.g. Valproic acid (VPA) and Carbamazepine (CBZ)) and the susceptibility for epilepsy development in the Jordanian population.

2. Methodology

2.1. Participants and data source

A cohort consisted of 296 unrelated epileptic patients and 299 healthy individuals participated in a pharmacogenetic and case-control study conducted between 2017 and 2018. This study was approved by Jordan University of Science and Technology with ethical approval number 16/111/2017. Samples were collected from the pediatric neurology clinic at Queen Rania Al Abdullah Hospital (QRAH) and the blood bank at the Jordanian Royal Medical Services (JRMS). A written informed consent was obtained from all participants in the study. Patients were included in this study if they were under the age of 15 years old, had at least two attacks of seizure within 24 h period of less than six months, received AEDs for at least three months prior to the study and had a normal psychomotoric development, neurologic examination and background activity. Patients were excluded if they lacked medical records, refused to provide written consent, had no reliable seizure frequency, refused to complete their AED treatment and suffered from liver disorders (Fig. 1).

2.2. Treatment approach

Antiepileptic protocol began with 10 mg/kg of valproic acid (VPA) for patients diagnosed with generalized seizure or 5 mg/kg daily of carbamazepine (CBZ) for patients diagnosed with partial seizure. After initiation of the therapy, seizure frequency was monitored for the first three to four weeks to ensure the dose effectiveness. In order to minimize the seizure frequency during the follow-up visits, the initial therapeutic dose was increased into 20 mg/kg and 10 mg/kg for the VPA and CBZ respectively. Based on their responsiveness to AEDs, patients were divided into good and poor responders (Fig. 1). Patients who require the lowest AED doses or who have taken only one drug without relapse in the past six months were considered as good responder patients (n = 162; 54.7%). In contrast, poor responders are those who require the highest AED doses or have taken more than one drug (n = 134; 45.3%). Patients' demographic and clinical data were summarized
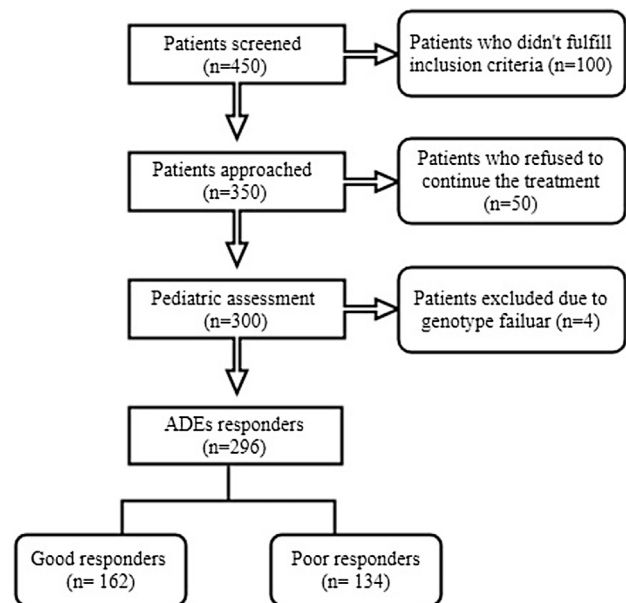

Fig. 1. Flowchart of epileptic patients' selection (n = 296).

in Supplementary Table 1 with no differences between patients in terms of age, gender, age of onset, and in subgroup distribution.

2.3. SNP selection and genetic analysis

DNA was extracted from the blood samples using the Wizard Genomic DNA Purification Kit (Promega Corporation, USA). Seven SNPs within *SCN2A* (rs2304016), *SCN3B* (rs3851100) and *GRM4* (rs2029461, rs2451334, rs745501, rs2499697 and rs937039) genes were selected from public genome databases such as the SNP database of the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/SNP/) and Ensemble database (http://www.ensembl.org/index.html). The chosen SNPs were selected based on their range of clinical relevance, reported functions and previously reported associations with responsiveness to treatment using the candidate SNP approach (Parihar et al., 2014; Haerian et al., 2013; Lakhan et al., 2009; Moldrich et al., 2003). Samples were sent to the Australian Genome Research Facility (AGRF, Australia) for SNPs genotyping with the Sequenom MassAR-RAY® system (iPLEX GOLD) (Sequenom, San Diego, CA, USA) according to the manufacturer's recommendations.

2.4. Clinical and phenotypic data

Association of clinical characteristics such as epilepsy syndromes, history of febrile seizure, psychosis, suicidal thoughts or actions, response to AEDs and family history of epilepsy in the 296 patients was assessed by clinical neurologists who were blind to the genotype results.

2.5. Statistical analysis

Different statistical genetic association analyses were performed to test the genetic association of the chosen SNPs with epilepsy susceptibility and responsiveness to AEDs treatment. The estimated genotype frequencies are calculated using Pearson's chi-squared test (χ^2). The minor allele frequency (MAF) and the HWE values for the genotype distribution were calculated according to the Court lab HW calculator. The SNPStats web tool (https://www.snpstats.net/start.htm) and the Statistical Package for Social

Sciences (SPSS) software (v. 22) were used to conduct all statistical analyses. P-value <0.05 was considered to be statistically significant.

3. Results

3.1. Allelic and genotypic distribution in epileptic patients

Epileptic and healthy individuals showed no significant differences in all studied SNPs within SCN2A, SCN3B and GRM4 genes at the single marker level (Table 1). However, after analysing the frequencies of GRM4 genetic haplotypes, we found that CAACG was significantly associated with epilepsy susceptibility (P = 0.036) (Table 2). The frequency of CAACG genetic haplotype were more frequent in epileptic patients than that in healthy individuals (2.93% vs. 0.79%, odds ratio (OR) = 3.53 (1.09–11.47)).

3.2. Association of SCN2A, SCN3B, and GRM4 SNPs with susceptibility to generalized epilepsy

Epileptic patients were further classified into those with generalized (GE) and partial (PE) epilepsy. SCN2A rs2304016 and GRM4 rs2499697 were of significant associations with GE (P = 0.044 and 0.031, respectively) (Table 3). The frequency of the SCN2A rs2304016 AG genotype was higher in GE patients than that in healthy controls (1.2% vs. 0.0%) (Table 3). Moreover, using the dominant genetic model CC/(CA + AA) of the GRM4 rs2499697 showed that the frequency of CC dominant genotype in GE patients (87.8%) was lower than healthy individuals (93.7%). In addition, GRM4 haplotypes analysis revealed strong association of the TGTAA block with GE (P = 0.006) (Table 4). The frequency of this genetic haplotype was more frequent in GE patients than that in healthy individuals (1.7% vs. 0.33%, odds ratio (OR) = 3.43 (1.42–8.83)).

3.3. Association of SCN2A, SCN3B, and GRM4 SNPs with partial epilepsy susceptibility

In case of the 124 partial epilepsy patients, genetic association analyses of the 7 SNPs revealed no significant differences in the PE and the healthy controls with p value more than 0.05 (Table 5). However, after conducting haplotype genetic analyses, this study found that CAACG haplotype of GRM4 gene was significantly asso-

ciated with PE (P = 0.024) (Table 6). The frequency of this haplotype was more frequent in patients than that in healthy individuals (4.2% vs. 0.8%, odds ratio (OR) = 4.00 (1.21–13.22)).

3.4. Association of SCN2A, SCN3B, and GRM4 SNPs genotypes with drug responsiveness

In term of drug responsiveness, we investigated the distribution of the 7 SNPs in poor and good responder patients. The frequency of the GRM4 rs2451334 GG genotype was higher in good responder than in poor responder [67.9% versus 57.5%, OR = 1.57 (0.97–2.52), P = 0.064] (Table 7) with no significant association of the GRM4 haplotypes with drug responsiveness in both responder groups with p-value more than 0.05 (Table 8).

3.5. Genotype- phenotype correlations

No clinical characteristics had been associated with studied genetic markers within SCN2A, SCN3B and GRM4 genes except for the rs2029461 and rs2451334 of GRM4 gene (Table 9). rs2029461 had a significant association with the family history of epilepsy (P = 0.015), where rs2451334 was associated with responsiveness to the AEDs treatment (P = 0.033) (Table 9).

4. Discussion

Any genes causing epilepsy that do not encode actual AED targets are also potential candidates for genetic variation in drug responsiveness to treatment (Spear, 2001; Depondt and Shorvon, 2006). Drugs interact with gene products (e.g. sodium, calcium ion channel and GABA receptor) that play a role in the etiology of the disease as well as the class of genes causing epilepsy which overlaps with common AED targets (Depondt, 2008; Ferraro and Buono, 2005). Several animal model studies indicated that mutations in genes causing epilepsy have demonstrated changes in response to several AEDs (Lucas et al., 2005). CBZ is one of the favorable drugs for treating partial seizure in the developed countries which affects Nav channels and inhibits rapid firing of brain cells (Fisher, 2010). Another AED is VPA, a standard broad-spectrum drug that is used to treat all types of seizures especially generalized seizure subtype (Fisher, 2010). Currently, AEDs such as carbamazepine, valproic acid, phenytoin, and lamotrigine act by

Table 1
The distributions of SCN2A, SCN3B and GRM4 SNPs in 296 epileptic patients and 299 healthy controls.

Genes	SNP	Model	Epilepsy patients %	Control %	P-value[*]
SCN2A	rs2304016	AA/AG	99.3/100	0.7/0.0	0.094
SCN3B	rs3851100	TT/TC/CC	85.3/14.7/0.0	82.3/17.1/0.7	0.18
		TT/(TC + CC)	85.3/14.7	82.3/17.7	0.31
		(TT + TC)/CC	100/0.0	99.3/0.7	0.098
GRM4	rs2029461	CC/CT/TT	32.3/41.9/25.8	29.9/45.3/24.8	0.7
		CC/(CT + TT)	32.3/67.7	29.9/70.1	0.52
		(CC + TC)/TT	74.2/25.8	75.2/24.8	0.79
	rs2451334	GG/GA/AA	63.2/31.4/5.4	63.4/31.2/5.4	1
		GG/(GA + AA)	63.2/36.8	63.4/36.6	0.95
		(GG + GA)/AA	94.6/5.4	94.6/5.4	0.98
	rs745501	AA/TA/TT	52.7/36.5/10.8	55.5/37.5/7	0.26
		AA/(TA + TT)	52.7/47.3	55.5/44.5	0.49
		(AA + TA)/TT	89.2/10.8	93.7	0.1
	rs2499697	CC/CA/AA	89.5/10.1/0.3	93.7/6.3/0.0	0.12
		CC/(CA + AA)	89.5/10.5	93.7/6.3	0.069
		(CC + CA)/AA	99.7/0.3	100/0.0	0.24
	rs937039	AA/AG/GG	37.2/48.3/14.5	42.8/41.5/15.7	0.24
		AA/(AG + GG)	37.2/62.8	42.8/57.2	0.16
		(AA + GA)/GG	85.5/14.5	84.3/15.7	0.68

[*] Chi-Square Test with P-value < 0.05 is considered significant.

Table 2
Frequencies of GRM4 haplotypes in 296 epileptic patients and 299 healthy controls.

Gene	Haplotypes	EP (%)	Controls (%)	Odd ratio (95% CI)	P-value[*]
GRM4	TGACG	0.319	0.3341	1.00	–
	CGACA	0.2118	0.2338	0.96 (0.70–1.32)	0.82
	CATCA	0.1504	0.1685	0.96 (0.68–1.36)	0.82
	TGACA	0.1095	0.1236	0.96 (0.64–1.44)	0.86
	CGTCA	0.0721	0.0481	1.41 (0.84–2.38)	0.2
	CAACG	0.0293	0.0079	3.53 (1.09–11.47)	0.036
	CGACG	0.0242	0.0086	2.23 (0.72–6.86)	0.16
	TGTCA	0.0174	0.0139	1.25 (0.47–3.32)	0.66
	CGAAA	0.0084	0.0174	0.49 (0.14–1.69)	0.26
	CATAA	0.0191	0.0045	3.17 (0.79–12.64)	0.1
	CAACA	0.0045	0.0158	0.21 (0.04–1.02)	0.054
	CATCG	0.0079	0.0125	0.67 (0.17–2.66)	0.57
	TGTAA	0.0166	0.0033	3.84 (0.74–19.94)	0.11

Global haplotype association P-value: 0.013.
[*] Chi-Square Test with P-value < 0.05 is considered significant.

Table 3
The distributions of SCN2A, SCN3B and GRM4 SNPs in 172 generalized epileptic patients and 299 healthy controls.

Genes	SNP	Model	Generalized patients %	Control %	P-value[*]
SCN2A	rs2304016	AA/AG	98.8/1.2	100/0.0	0.044
SCN3B	rs3851100	TT/TC/CC	85.5/14.5/0.0	82.3/17.1/0.7	0.3
		TT/(TC + CC)	85.5/14.7	82.3/17.7	0.18
		(TT + TC)/CC	100/0.0	99.3/0.7	0.47
GRM4	rs2029461	CC/CT/TT	32.4/42.9/24.7	29.9/45.3/24.8	0.84
		CC/(CT + TT)	32.4/67.7	29.9/70.1	0.58
		(CC + TC)/TT	75.3/24.7	75.2/24.8	0.98
	rs2451334	GG/GA/AA	66.3/29.1/4.7	63.4/31.2/5.4	0.81
		GG/(GA + AA)	63.3/33.7	63.4/36.6	0.53
		(GG + GA)/AA	95.3/4.7	94.6/5.4	0.73
	rs745501	AA/TA/TT	51.7/37.2/11.1	55.5/37.5/7	0.32
		AA/(TA + TT)	51.7/48.3	55.5/44.5	0.43
		(AA + TA)/TT	89/11.1	93/7	0.14
	rs2499697	CC/CA/AA	87.8/11.6/0.6	93.7/6.3/0.0	0.051
		CC/(CA + AA)	87.8/12.2	93.7/6.3	0.031
		(CC + CA)/AA	99.4/0.6	100/0.0	0.16
	rs937039	AA/AG/GG	37.2/47.1/15.7	42.8/41.5/15.7	0.44
		AA/(AG + GG)	37.2/62.8	42.8/57.2	0.23
		(AA + GA)/GG	84.3/15.7	84.3/15.7	1

[*] Chi-Square Test with P-value < 0.05 is considered significant.

Table 4
Frequencies of GRM4 haplotypes in 172 generalized epileptic patients and 299 healthy controls.

Gene	Haplotypes	GEP (%)	Controls (%)	Odd ratio (95% CI)	P-value[*]
GRM4	TGACG	0.3176	0.3341	1.00	–
	CGACA	0.2026	0.2338	0.90 (0.62–1.31)	0.6
	CATCA	0.1382	0.1685	0.86 (0.57–1.32)	0.5
	TGACA	0.1004	0.1236	0.87 (0.53–1.42)	0.59
	CGTCA	0.0858	0.0481	1.70 (0.94–3.07)	0.079
	CGACG	0.0347	0.0086	3.20 (1.01–10.19)	0.05
	CGAAA	0.0152	0.0174	0.88 (0.26–2.99)	0.84
	TGTCA	0.02	0.0139	1.39 (0.48–4.07)	0.54
	CAACA	0.0069	0.0158	0.33 (0.07–1.61)	0.17
	CAACG	0.0215	0.0079	2.81 (0.72–10.99)	0.14
	CATCG	0.0084	0.0125	0.69 (0.14–3.43)	0.65
	TGTAA	0.017	0.0033	3.54 (1.42–8.83)	0.0069

Global haplotype association P-value: 0.0093.
[*] Chi-Square Test with P-value < 0.05 is considered significant.

blocking the neuronal Nav channel or by enhancing inhibitory GABAergic neurotransmission (Moldrich et al., 2003; Szoeke et al., 2006). Nav channels are the primary sites of action for many AEDs andthe inactivation of these channels is the primary mechanism to prevent epileptic seizures (Kwan et al., 2008). Variation in drug response or failure to control the seizure may arise from genetic polymorphisms in Nav channel genes (Haerian et al., 2013; Kwan et al., 2008). These channels are composed of α and β subunit which encoded by Nav channel genes such as SCN2A and SCN3B (Kwan et al., 2008; Goldin et al., 2000). Moreover, Glutamate is the principal excitatory neurotransmitter in the mammalian brain and exerts its effect through one of the glutamate receptors in the CNS such as mGluR (Meldrum, 2000). Based on neurophysiological and pharmacological studies, mGluR 4 is a

Table 5
The distributions of SCN2A, SCN3B and GRM4 SNPs in 124 partial epileptic patients and 299 healthy controls.

Genes	SNP	Model	PE patients %	Control %	P-value[*]
SCN3B	rs3851100	TT/TC/CC	85.1/14.9/0.0	82.3/17.1/0.7	0.43
		TT/(TC + CC)	85.1/14.9	82.3/17.7	048
		(TT + TC)/CC	100/0.0	99.3/0.7	0.24
GRM4	rs2029461	CC/CT/TT	32.2/40.5/27.3	29.9/45.3/24.8	0.67
		CC/(CT + TT)	32.2/67.8	29.9/70.1	0.63
		(CC + CT)/TT	72.7/27.3	75.2/24.8	0.61
	rs2451334	GG/GA/AA	58.9/34.7/6.5	63.4/31.2/5.4	0.67
		GG/(GA + AA)	58.9/41.1	63.4/36.6	0.38
		(GG + GA)/AA	93.5/6.5	94.6/5.4	0.67
	rs745501	AA/TA/TT	54/35.5/10.5	55.5/37.5/7	0.5
		AA/(TA + TT)	54/46	55.5/44.5	0.78
		(AA + TA)/TT	89.5/10.5	93.7	0.24
	rs2499697	CC/CA	91.9/8.1	93.7/6.3	0.53
	rs937039	AA/AG/GG	37.1/50/12.9	42.8/41.5/15.7	0.27
		AA/(AG + GG)	37.1/62.9	42.8/57.2	0.28
		(AA + GA)/GG	87.1/12.9	84.3/15.7	0.45

[*] Chi-Square Test with P-value < 0.05 is considered significant.

Table 6
. Frequencies of GRM4 haplotypes in 121 partial epileptic patients and 297 healthy controls.

Gene	Haplotypes	PEP (%)	Controls (%)	Odd ratio (95% CI)	P-value[*]
GRM4	TGACG	0.3195	0.3341	1.00	–
	CGACA	0.2303	0.2337	1.07 (0.72–1.61)	0.73
	CATCA	0.1578	0.1667	1.07 (0.69–1.68)	0.76
	TGACA	0.1243	0.1244	1.11 (0.66–1.86)	0.69
	CGTCA	0.0561	0.048	1.09 (0.53–2.25)	0.81
	CAACG	0.042	0.008	4.00 (1.21–13.22)	0.024
	TGTCA	0.0142	0.014	1.13 (0.28–4.54)	0.87
	CGAAA	NA	0.0175	0.00 (−Inf – Inf)	1
	CAACA	NA	0.0158	0.00 (−Inf – Inf)	1
	CATCA	0.0075	0.0125	3.17 (0.79–12.64)	0.1
	CAACA	0.0045	0.0158	0.21 (0.04–1.02)	0.054
	CATCG	0.0079	0.0125	0.67 (0.17–2.66)	0.57
	TGTAA	0.0166	0.0033	3.84 (0.74–19.94)	0.11

Global haplotype association P-value: 0.03.
[*] Chi-Square Test with P-value < 0.05 is considered significant.

Table 7
The distributions of SCN2A, SCN3B and GRM4 SNPs in 134 poor responder patients and 162 good responder patients.

Genes	SNP	Model	Poor responders %	Good responders %	P-value[*]
SCN2A	rs2304016	AA/AG	99.2/0.8	99.4/0.6	0.89
SCN3B	rs3851100	TT/TC	85.8/14.2	84.9/15.1	0.83
GRM4	rs2029461	CC/CT/TT	36.4/43.2/20.4	28.9/40.9/30.2	0.13
		CC/(CT + TT)	36.4/63.6	28.9/71.1	0.18
		(CC + TC)/TT	79.5/20.4	69.8/30.2	0.057
	rs2451334	GG/GA/AA	57.5/35.8/6.7	67.9/27.8/4.3	0.17
		GG/(GA + AA)	57.5/42.5	67.9/32.1	0.064
		(GG + GA)/AA	93.3/6.7	95.7/4.3	0.37
	rs745501	AA/TA/TT	51.5/36.6/11.9	53.7/36.4/9.9	0.84
		AA/(TA + TT)	51.5/48.5	53.7/46.3	0.7
		(AA + TA)/TT	88.1/11.9	90.1/9.9	0.57
	rs2499697	CC/CA/AA	89.5/9.7/0.8	89.5/10.5/0.0	0.44
		CC/(CA + AA)	89.5/10.4	89.5/10.5	0.99
		(CC + CA)/AA	99.2/0.8	100/0.0	0.21
	rs937039	AA/AG/GG	38.1/47/14.9	36.4/49.4/14.2	0.92
		AA/(AG + GG)	38.1/61.9	36.4/63.6	0.77
		(AA + GA)/GG	85.1/14.9	85.8/14.2	0.86

[*] Chi-Square Test with P-value < 0.05 is considered significant.

presynaptic receptor that inhibits the release of glutamate and GABA from nerve terminal sites with an indicated role in the epileptogenesis (Izzi et al., 2003). Several experimental seizure models have demonstrated abnormal glutamate receptor function which implicated in the initiation and spreading of the seizure (Meldrum, 1995; Meldrum and Rogawski, 2007; Chapman, 2000).

mGluR ligands are relatively novel compared to the Nav channel inhibitors and their biological mechanism to control epileptic seizures has not yet been fully explored in the scientific literature (Moldrich et al., 2003). Limited information about these genes and their association were mentioned in the scientific literature. The novelty of this study lies in the hypothesis being tested where

Table 8
Frequencies of GRM4 haplotypes in 134 poor responders and 162 good responders.

Gene	Haplotypes	Poor responder %	Good responder %	Odd ratio (95% CI)	P-value[*]
GRM4	TGACG	0.3053	0.3279	1.00	–
	CGACA	0.2415	0.1898	1.31 (0.81–2.12)	0.27
	CATCA	0.1695	0.1423	1.29 (0.77–2.16)	0.33
	TGACA	0.0765	0.1388	0.65 (0.35–1.19)	0.17
	CGTCA	0.063	0.0759	0.79 (0.40–1.57)	0.51
	CAACG	0.0361	0.0171	2.10 (0.65–6.78)	0.21
	CGACG	0.0233	0.0252	1.01 (0.31–3.24)	0.99
	CATAA	0.0276	0.0049	0.94 (0.27–3.29)	0.92
	TGTCA	0.0158	0.0183	1.10 (0.31–3.97)	0.88
	TGTAA	0.0159	0.0156	4.12 (0.50–34.11)	0.19

Global haplotype association P-value: 0.34.
[*] Chi-Square Test with P-value < 0.05 is considered significant.

Table 9
Genotype- phenotype association of SCN2A, SCN3B and GRM4 SNPs.

Clinical characteristics	Genes and SNPs						
	SCN2A	SCN3B	GRM4				
	rs2304016	rs3851100	rs2029461	rs2451334	rs745501	rs2499697	rs937039
Epilepsy syndromes	0.828	0.787	0.435	0.692	0.508	0.591	0.243
History of febrile seizure	0.757	0.203	0.229	0.192	0.140	0.684	0.764
Psychosis	0.908	0.713	0.668	0.063	0.646	0.134	0.950
Suicidal thoughts or actions	0.970	0.739	0.653	0.975	0.920	0.940	0.815
Response to AEDs[*]	0.088	0.729	0.059	**0.033**	0.166	0.096	0.168
Family history of epilepsy	0.969	0.740	**0.015**	0.974	0.917	0.611	0.067

[*] AEDs: Antiepileptic treatment protocol began with 10 mg/kg of valproic acid (VPA) for patients diagnosed with generalized seizure or 5 mg/kg daily of carbamazepine (CBZ) for patients diagnosed with partial seizure. Chi-Square Test with P-value < 0.05 is considered significant.

few or no studies have been conducted to identify genetic markers in order to predict responsiveness to AED treatment and epilepsy prognosis in Jordanian population. Different type of studies on populations from Hong Kong, Malaysia and China found no significant association of polymorphisms in the SCN2A gene with AEDs responsiveness in epileptic patients (Haerian et al., 2013; Zhou et al., 2015). In contrast, Kwan et al. and Li et al. suggested that treatment responsiveness was significantly associated with the genetic polymorphism within SCN2A gene (Kwan et al., 2008; Li et al., 2016). A study on north Indian population indicates a differential role of SCN2A gene in epilepsy susceptibility and drug responsiveness to treatment (Lakhan et al., 2009). In our results, we found that SCN2A variant has been associated with the susceptibility of generalized epilepsy but not with the treatment responsiveness in epileptic patients. In a five-generational Chinese epileptic family with generalized seizure, SCN3B gene was not associated with seizure susceptibility (Lu et al., 2010). Inconsistent, the dysregulation of SCN3B gene might play an important role in the progression of epilepsy and a potential target for epilepsy treatment (Jin et al., 2016). Furthermore, the current study found no significant associations in the SCN3B gene of Nav channel with epilepsy susceptibility and AEDs treatment response and in Jordanian population. A case–control study was carried out to check the association of GRM4 gene locus with idiopathic form of epilepsy in an Indian population. Only one out of the 5 tested SNPs was significantly associated with epilepsy susceptibility which is further strengthened by haplotype analysis (Parihar et al., 2014). Izzi et al. (2003) reported that there is genetic association between epilepsy susceptibility and GRM4 gene common variants in German population (Izzi et al., 2003). Taken together, our results indicated that there is no genetic association between GRM4 polymorphisms and responsiveness to the treatment or epilepsy susceptibility except for one SNP that found to be statistically associated with GE compared to the healthy individuals. For further observation,

the association of genetic haplotypes was conducted and revealed a transmission of risk alleles in epilepsy as well as in generalized and partial epilepsy probands. These differences in the associations of the gene variants and haplotypes could be explained by the ethnicity and genetic backgrounds diversity. GRM4 rs2451334 showed statistically significant association with AEDs treatment response suggesting its importance in the pharmacotherapy of epilepsy. In addition, rs2029461 was associated with the family history of epilepsy which could predict the inheritance nature of this disease.

5. Conclusion

In summary, our results suggest that SCN2A, SCN3B and GRM4 genes variant were not associated with neither epilepsy susceptibility nor drug responsiveness in Jordanian patients with epilepsy. Nevertheless, generalized epilepsy type has been associated in patients with SCN2A rs2304016 and GRM4 rs2499697 variants. Further investigations with a larger sample size and inclusion of other polymorphisms of candidate genes will improve disease diagnosis and optimize the AEDs treatment in order to reduce the risk of seizure attack events which mostly associated with inappropriate dose.

Ethical approval and consent to participate

This study was approved by the institutional Review Board (IRB) at Jordan University of Science and Technology with ethical approval number 16/111/2017. A written informed consent was obtained from all participants in the study. This study was also performed in accordance with the Declaration of Helsinki 1975, as revised in 2013.

Acknowledgments

Conflict of interest

The Authors declare no conflict of interest, financial or otherwise.

Appendix A. Supplementary material

Demographic and clinical data of the 296 participated epileptic patients were tabulated in Supplementary Table 1. Supplementary data to this article can be found online at https://doi.org/10.1016/j.jsps.2019.04.009.

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