Neurological Sciences (2019) 40:523–528
https://doi.org/10.1007/s10072-018-3681-y

ORIGINAL ARTICLE

Lack of association between valproic acid response and polymorphisms of its metabolism, transport, and receptor genes in children with focal seizures

Weixing Feng[1,2] · Shenghui Mei[3,4] · Jiaqi Han[3,4] · Leting Zhu[3] · Yazhen Yu[2] · Baoqin Gao[2] · Yun Wu[1] · Jiuwei Li[1] · Zhigang Zhao[3,4] · Fang Fang[1]

Received: 11 June 2018 / Accepted: 10 December 2018 / Published online: 18 December 2018

Abstract

Objective This study aims to describe the associations between genetic polymorphisms and therapeutic effect of valproic acid (VPA) in children with focal seizures.

Methods Eighty-nine children with focal seizures on VPA therapy were enrolled. Patients' basic information, dosage regimens, and plasma concentrations were recorded. A 1-year follow-up was performed to evaluate the treatment response. Sixty-six single nucleotide polymorphisms involved in the metabolism, transport, and target receptor of VPA were identified, and their associations with VPA response were analyzed using logistic regression adjusted by various influence factors. Selected polymorphisms involved in the metabolism, transport, and target receptor of VPA were not associated with treatment effect in children with focal seizures.

Results Three variants, rs9313892 (*GABRA6*, G > A, OR = 2.73, 95% CI 1.00 to 7.48, $P = 0.051$), rs4921195 (*GABRA6*, T > C, OR = 2.71, 95% CI 0.99 to 7.42, $P = 0.053$), and rs424740 (*GABRG2*, A > T, OR = 0.39, 95% CI 0.15 to 1.01, $P = 0.053$) had the potential to be associated with the VPA response.

Conclusion Selected genetic polymorphisms were not significantly associated with VPA response in children with focal seizures. However, three *GABR* variants showed potential to be associated with the response to VPA. Further and larger studies are warranted to confirm the results.

Keywords Children · Valproic acid · Genetic polymorphisms · Drug-resistant epilepsy · Focal seizures

Abbreviations

VPA	Valproic acid
ABCB1	ATP binding cassette subfamily B member 1
ABCC2	ATP binding cassette subfamily C member 2

Weixing Feng, Shenghui Mei, and Jiaqi Han are equal first authors

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10072-018-3681-y) contains supplementary material, which is available to authorized users.

✉ Zhigang Zhao
ttyyzzg1022@126.com

✉ Fang Fang
13910150389@163.com

[1] Department of Neurology, Beijing Children's Hospital, Capital Medical University, Beijing, People's Republic of China

[2] Department of Pediatrics, Beijing Tiantan Hospital, Capital Medical University, Beijing, People's Republic of China

[3] Department of Pharmacy, Beijing Tiantan Hospital, Capital Medical University, Beijing, People's Republic of China

[4] Department of Clinical Pharmacology, College of Pharmaceutical Sciences, Capital Medical University, Beijing, People's Republic of China

GABA	Gamma-aminobutyric acid
ABAT	4-Aminobutyrate aminotransferase
GABR	GABA receptor
GABRG2	GABA type A receptor gamma 2 subunit
SCN	Sodium voltage-gated channel
NMDA	N-methyl-D-aspartate
GRIN	Glutamate ionotropic receptor NMDA type
MAF	Minor allele frequency
HWE	Hardy–Weinberg equilibrium
OR	Odds ratio
95% CI	95% confidence interval
GABRA6	Gamma-aminobutyric acid type A receptor alpha 6 subunit
CYP2C9	Cytochrome P450 family 2 subfamily C member 9.

Introduction

Valproic acid (VPA) has been widely used to treat children with various types of seizures. However, VPA response and its toxicities varied greatly among individuals [1, 2], which could be attributed to the various genetic polymorphisms involved in the metabolism, transport, and target receptor of VPA [3]. In adults, glucuronidation and mitochondrial β-oxidation were two major pathways in VPA metabolism [4]. The glucuronidase activity was age-dependent, but their quantitative relationships were not clarified [5]. Children have a lower glucuronidase activity than adults, therefore, the mitochondrial β-oxidation was supposed to be the major pathway for VPA metabolism in children [6]. Clinical studies indicated that genetic polymorphisms involved in the mitochondrial β-oxidation and glucuronidation of VPA were associated with its plasma concentration and clearance [7–11]. To exhibit the anticonvulsant activity, VPA needs to be transported to the target site by various transporters, such as ATP binding cassette subfamily B member 1 (ABCB1) and ATP binding cassette subfamily C member 2 (ABCC2). Associations between polymorphisms of these transporters and drug resistance were observed in patients with epilepsy [12–15], but the results were not replicated in other studies [15–17].

The inhibitory neurotransmitter gamma-aminobutyric acid (GABA) is mainly metabolized by 4-aminobutyrate aminotransferase (ABAT), which could be inactivated by VPA [4]. Moreover, VPA positively regulated the function of the GABA receptor (GABR), then enhanced the receptor-mediated inhibition [18]. The protective effect of rs2279020 (G > A, GABA type A receptor alpha 1 subunit) in patients with epilepsy was reported [11, 19], but it was not verified in other studies [17]. rs211037 (C > T, GABA type A receptor gamma 2 subunit, *GABRG2*), an independent risk factor for febrile seizure, in linkage with rs210987 could contribute to febrile seizures and symptomatic epilepsy [20]. However, another study disclosed that rs211037 was associated with epilepsy susceptibility rather than antiepileptic drug resistance and febrile seizures [21].

The biological activity of VPA was partly attributed to the suppression on sodium voltage-gated channels which were encoded by the sodium voltage-gated channel (*SCN*) gene family [4, 18, 22]. VPA response was associated with various variants in the *SCN* gene family, such as rs3812718 [23] and rs2304016 [24, 25], but these results were not confirmed by the other studies for rs3812718 [25, 26] and rs2304016 [17]. Besides, VPA could affect neuronal excitation via regulation of the N-methyl-D-aspartate (NMDA) subtype of glutamate receptors [18, 22], which were encoded by the glutamate ionotropic receptor NMDA type (*GRIN*) gene family, some of which polymorphisms were associated with VPA concentration to dose ratio [7]. Furthermore, various concomitant antiepileptic drugs [27], body surface area [9], and alcohol and soybean intake [28, 29] could also affect VPA metabolism and response.

In our previous studies, children were grouped by VPA dosage form (oral solution or sustained tablet) to analyze the influence of genetic polymorphisms on VPA plasma concentration. To evaluate the influence of genetic polymorphisms on VPA efficacy, children were grouped by their seizure type (generalized or focal). There are overlaps of the patients between different groups. In children taking VPA oral solution, rs28898617 (*UGT1A6*, A > G) was associated with an increased VPA plasma concentration (normalized by body weight and total daily dose), and rs2279020 (*GABRA1*, G > A) was associated with a decreased risk to develop VPA-resistant epilepsy [11]. In children taken sustained VPA tablet monotherapy, rs28898617 was associated with higher logarithmic transformed VPA plasma concentration (normalized by body weight and total daily dose) [10]. For children with generalized seizures, rs7668282 (*UGT2B7*, T > C) was a risk factor while rs2242480 (*CYP3A4*, C > T) and rs10188577 (*SCN1A*, T > C) were protective factors for drug-resistant epilepsy [30]. Moreover, in our population pharmacokinetic model, total daily dose, body surface area, and a combined genotype of four variants (rs1042597, rs28365062, rs4986893, and rs4244285) could affect VPA clearance [9].

In this study, the associations between VPA treatment response and genetic polymorphisms involved in VPA metabolism, transport, and targeting were analyzed in children with focal seizures.

Methods

Study design

This study was approved by the Ethics Committee of Beijing Tiantan Hospital, Capital Medical University, Beijing, China. Signed informed consents were obtained from all patients or

their direct relatives. Inclusion criteria: (1) met the diagnostic criteria of focal seizures [31], (2) aged 2 to 18 years, (3) on VPA therapy, (4) Han Chinese, (5) without other serious diseases. Exclusion criteria were severe renal or liver impairment and failure to get the efficacy data.

After enrollment, patients' information including demographic data, dosage regimens, seizure type [32], and seizure frequency (before drug intake and after 1-year treatment) were recorded. A reduction in seizure frequency of less than 50% was used to define drug resistance [11, 33]. After regularly taking VPA for more than 30 days, the blood samples of patients were collected (within 2 h before the next dose), then after a 10 min centrifugation at 5000*g*, the supernatants of the samples were used to analyze the VPA concentration, while the white blood cells were stored at − 80 °C for genotyping by MassArray system. A 1-year follow-up was performed to evaluate the therapeutic effect of VPA. The associations between genetic polymorphisms and therapeutic response of VPA were analyzed using logistic regression adjusted by age, gender, body weight, dosing frequency, total daily dose, dosage form, VPA plasma concentration, and concomitant antiepileptic drugs to remove their influence.

Plasma concentration of VPA

VPA plasma concentration was evaluated by fluorescence polarization immunoassay (TDx, ABBOTT, USA). According to the kit instructions, calibration and quality control samples were routinely performed with acceptable results.

Genotyping

Patients' DNA was purified by Qiagen DNA purification kit (Qiagen, Hilden, Germany). Genotyping was identified by MassArray method (Sequenom, USA) in Bio Miao Biological Technology (Beijing) [34]. Five percent of the whole samples were measured twice for quality control with acceptable reproducibility.

Statistical analysis

SPSS (version 17.0, SPSS Inc., Chicago, USA) and PLINK (version 1.07, Shaun Purcell, Boston, USA) software were used for statistical analysis. Continuous variables were analyzed by Student's *t* test or nonparametric test. The classified variables were analyzed by the chi-square test. The associations between genetic polymorphisms and therapeutic effect were evaluated using logistic regression adjusted by various factors (PLINK software). The Minor Allele Frequency (MAF), Hardy–Weinberg equilibrium test (HWE, *P* value for chi-square test), odds ratio (OR), and its 95% confidence interval (95% CI), and *P* value (asymptotic *P* value for *z*-statistic) were calculated [35]. Statistical significance was defined as a *P* value less than 0.05.

Results

Characteristics of enrolled patients

Eighty-nine children (58 males and 31 females) with focal seizures were enrolled. Patients' clinical characteristics are summarized in Table 1 (detail in Appendix 1). The values of all variables were similar between drug-responsive (68 patients) and drug-resistant groups (21 patients).

Genotyping and its association with therapeutic effect

The information of selected variants are shown in Appendix 1. All variants were in accordance with the HWE ($P > 0.05$). None of the variants was significantly associated with the VPA effect. Three variants, rs9313892 (gamma-aminobutyric acid type A receptor alpha 6 subunit, *GABRA6*, G > A, OR = 2.73, 95% CI 1.00 to 7.48, $P = 0.051$), rs4921195 (*GABRA6*, T > C, OR = 2.71, 95% CI 0.99 to 7.42, $P = 0.053$), and rs424740 (*GABRG2*, A > T, OR = 0.39, 95% CI 0.15 to 1.01, $P = 0.053$) had the potential to be associated with the therapeutic effect (Table 2, detail in Appendix 2).

Discussion

Mitochondrial β-oxidation might be the major pathway in VPA metabolism in children [6]. Cytochrome P450 family 2 subfamily C member 9 (CYP2C9)-status guided therapy has been successfully applied for VPA dose optimization to avoid its adverse reactions in Caucasian children [6]. The MAFs of *CYP2C9*2* (rs1799853) and *CYP2C9*3* (rs1057910) were very low in Han Chinese (0 and 0.039, data from the 1000genomics). In the present study, *CYP2C9*2* was not studied, and *CYP2C9*3* was not associated with VPA response due to its low MAF (0.017).

Polymorphisms of various transporters such as *ABCB1* and *ABCC2* have been associated with drug-resistant epilepsy [12–14]. However, in accordance with many previous studies [15–17], these associations were not found in the present study. The following reasons might be used to explain the contradictory results [36]: the functional alterations of important variants of these transporters (such as *ABCB1* C3435T polymorphism) have not been identified [13]; multidrug-resistant transporters have been found to be expressed in the blood-brain and blood-cerebrospinal fluid barriers [37], however, the contribution of these transporters for VPA transport was not established clearly,

Table 1 Clinical characteristics of enrolled patients

Variable	Children with focal seizures ($n = 89$)		
	Response ($n = 68$)	Resistant ($n = 21$)	P value
Gender (male/female)	45/23	12/9	0.450
Age (year)	8.66 ± 3.94 (2–16)	7.07 ± 3.79 (2–14)	0.107
Body weight (kg)	32.72 ± 15.62 (12–80)	26.51 ± 10.9 (14.5–50)	0.114
Dosage form (oral solution/sustained tablet)	23/45	8/13	0.719
Dosing frequency (qd/bid/tid)	5/53/10	1/16/4	0.836
Total daily dose (mg)	591.5 ± 241.84 (120–1000)	678.45 ± 290.57 (360–1500)	0.301
Plasma concentration of VPA (mg/L)	54.32 ± 24.5 (11.69–108.94)	48.99 ± 26.39 (0.21–121.22)	0.395
Antiepileptic comedications			
Lamotrigine	2	4	
Levetiracetam	4	1	
Carbamazepine	4	2	
Oxcarbazepine	7	3	
Clonazepam	1	0	
Phenobarbital	2	0	
Phenytoin	0	1	
Nitrazepam	1	0	

and the expression and function of these transporters have not been identified in these studies; different members of the transporters have been shown to be distributed in different regions of the brain [38], but the contribution of these transporters on drug-resistant epilepsy was not explained; seizures themselves and some antiepileptic drugs could induce multidrug-resistant genes and result in an upregulation of some multidrug transporters in animals [38], but the extent of their importance remains unknown.

Sixteen variants of *GABR* family and two variants of *ABAT* were analyzed in our patients, but none of them was associated with VPA response as reported in previous studies [17, 39]. However, two variants of *GABRA6* (rs9313892 and rs4921195) and one variant of *GABRG2* (rs424740) had the potential to be associated with VPA response (P values close to 0.05, Table 2). Both two intron variants of *GABRA6* were located at the 5′ flanking with unknown function. There was a lack of studies about the associations between these two variants and VPA response. A study indicated that rs424740 was not a major factor in the pathogenesis of mesial temporal lobe epilepsy in the Indian population [40]. The frequency of rs424740 allele A has been reported to be 0.67 for Han Chinese, 0.31 for American, and 0.39 for European populations (data from 1000genomics database, available at http://grch37.ensembl.org/Homo_sapiens/Variation/Population?db=core;r=5:161580535-161581535;v=rs424740;vdb=variation;vf=280184). The exact effect of this variant on protein expression and function needs to be clarified in further investigations.

VPA response has been associated with various variants of the *SCN* gene family, such as rs3812718 [23] and rs2304016 [24]. In our patients, none of the selected variants of the *SCN* gene family was associated with VPA response as reported in previous researches [17, 25]. The difference of mutation frequency among different races, patients' basic information, and disease status in different studies might be explanations for the conflictive results.

Table 2 Information of variants with significant association with treatment response

7	SNP	Position	Allele (major > minor)	MAF	Genotype	HWE (P value)	OR, 95%CI	P value
GABRA6	rs9313892	5′ flanking	G > A	0.315	9/38/42	1	2.73, 1.00–7.48	0.051
GABRA6	rs4921195	5′ flanking	T > C	0.314	9/37/42	0.808	2.71, 0.99–7.42	0.053
GABRG2	rs424740	UTR-3	A > T	0.352	15/32/41	0.062	0.39, 0.15–1.01	0.053

SNP, single nucleotide polymorphism; *MAF*, minor allele frequency; *HWE*, Hardy–Weinberg equilibrium, P value of chi-square test; *OR*, odds ratio; *95%CI*, 95% confidence interval; *GABRA6*, gamma-aminobutyric acid type A receptor alpha 6 subunit; *GABRG2*, gamma-aminobutyric acid type A receptor gamma 2 subunit; *UTR-3*, untranslated region 3

In summary, none of the variants in various genes involved in the metabolism, transport, and target receptor of VPA was significantly associated with VPA response.

Deficiencies of the study

Limitations of the study: (1) the sample size was relatively small (89 patients); (2) variants with low frequency (MAF < 0.05) were not selected; (3) the influences of non-antiepileptic concomitant medications on VPA response were not considered despite their influence on VPA metabolism [27]; (4) many variants were not identified despite their influence on epilepsy response [41]; (5) the food-drug interaction on VPA metabolism and response was not considered [28, 29].

Conclusions

Therapeutic response was not significantly associated with selected genetic polymorphisms involved in the metabolism, transport, and target receptor of VPA in children with focal seizures. However, three *GABR* variants showed potential to be associated with the response to VPA. Further and larger studies are warranted to confirm the results.

Acknowledgments Thanks to our patients and our whole team. Particularly grateful to professor Shiqi Peng, Ming Zhao, Yuji Wang (College of Pharmaceutical Science, Capital Medical University, Beijing, China), Jiawang Liu (Medicinal Chemistry Core, Division of Vice Chancellor for Research, University of Tennessee Health Science Center), and the reviewers for their help in manuscript revision.

Author contributions Weixing Feng: study design, data analysis, follow-up, and manuscript revising.
Shenghui Mei: study design, data analysis, and manuscript revising.
Jiaqi Han: data analysis, follow-up, and manuscript revising.
Leting Zhu: sample collection and valproic acid plasma concentration analysis.
Yazhen Yu: patient's information collection and follow-up.
Baoqin Gao: study design and manuscript revising.
Yun Wu: patient's information collection and follow-up.
Jiuwei Li: patient's information collection and follow-up.
Zhigang Zhao: study design and manuscript revising.
Fang Fang: study design and manuscript revising.

Funding This study was funded by the National Natural Science Foundation of China (no. 81301118).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

References

1. Tomson T, Battino D, Perucca E (2016) Valproic acid after five decades of use in epilepsy: time to reconsider the indications of a time-honoured drug. Lancet Neurol 15(2):210–218
2. Dedei Daryan M, Guveli BT, Baslo SA, Mulhan K, Sari H, Balcik ZE, Atakli D (2018) Prevalence and clinical characteristics of headache in juvenile myoclonic epilepsy: experience from a tertiary epilepsy center. Neurol Sci 39(3):519–525
3. Steinlein OK (2010) Gene polymorphisms and their role in epilepsy treatment and prognosis. Naunyn Schmiedeberg's Arch Pharmacol 382(2):109–118
4. Ghodke-Puranik Y, Thorn CF, Lamba JK, Leeder JS, Song W, Birnbaum AK, Altman RB, Klein TE (2013) Valproic acid pathway: pharmacokinetics and pharmacodynamics. Pharmacogenet Genomics 23(4):236–241
5. de Wildt SN, Kearns GL, Leeder JS, van den Anker JN (1999) Glucuronidation in humans. Pharmacogenetic and developmental aspects. Clin Pharmacokinet 36(6):439–452
6. Budi T, Toth K, Nagy A, Szever Z, Kiss A, Temesvari M, Hafra E, Garami M, Tapodi A, Monostory K (2015) Clinical significance of CYP2C9-status guided valproic acid therapy in children. Epilepsia 56(6):849–855
7. Hung CC, Ho JL, Chang WL, Tai JJ, Hsieh TJ, Hsieh YW, Liou HH (2011) Association of genetic variants in six candidate genes with valproic acid therapy optimization. Pharmacogenomics 12(8): 1107–1117
8. Guo Y, Hu C, He X, Qiu F, Zhao L (2012) Effects of UGT1A6, UGT2B7, and CYP2C9 genotypes on plasma concentrations of valproic acid in Chinese children with epilepsy. Drug Metab Pharmacokinet 27(5):536–542
9. Mei S, Feng W, Zhu L, Li X, Yu Y, Yang W, Gao B, Wu X, Fang F, Zhao Z (2018) Effect of CYP2C19, UGT1A8, and UGT2B7 on valproic acid clearance in children with epilepsy: a population pharmacokinetic model. Eur J Clin Pharmacol 74(8):1029–1036
10. Mei S, Feng W, Zhu L, Yu Y, Yang W, Gao B, Wu X, Zhao Z, Fang F (2017) Genetic polymorphisms and valproic acid plasma concentration in children with epilepsy on valproic acid monotherapy. Seizure 51:22–26
11. Feng W, Mei S, Zhu L, Yu Y, Yang W, Gao B, Wu X, Zhao Z, Fang F (2016) Effects of UGT1A6 and GABRA1 on standardized valproic acid plasma concentrations and treatment effect in children with epilepsy in China. Ther Drug Monit 38(6):738–743
12. Qu J, Zhou BT, Yin JY, Xu XJ, Zhao YC, Lei GH, Tang Q, Zhou HH, Liu ZQ (2012) ABCC2 polymorphisms and haplotype are associated with drug resistance in Chinese epileptic patients. CNS Neurosci Ther 18(8):647–651
13. Siddiqui A, Kerb R, Weale ME, Brinkmann U, Smith A, Goldstein DB, Wood NW, Sisodiya SM (2003) Association of multidrug resistance in epilepsy with a polymorphism in the drug-transporter gene ABCB1. N Engl J Med 348(15):1442–1448
14. Chouchi M, Kaabachi W, Klaa H, Tizaoui K, Turki IB, Hila L (2017) Relationship between ABCB1 3435TT genotype and antiepileptic drugs resistance in epilepsy: updated systematic review and meta-analysis. BMC Neurol 17(1):32
15. Qian L, Fang S, Yan YL, Zeng SS, Xu ZJ, Gong ZC (2017) The ABCC2 c.-24C>T polymorphism increases the risk of resistance to antiepileptic drugs: a meta-analysis. J Clin Neurosci 37:6–14
16. Haerian BS, Lim KS, Mohamed EH, Tan HJ, Tan CT, Raymond AA, Wong CP, Wong SW, Mohamed Z (2011) Lack of association of ABCB1 haplotypes on five loci with response to treatment in epilepsy. Seizure 20(7):546–553
17. Zhou L, Cao Y, Long H, Long L, Xu L, Liu Z, Zhang Y, Xiao B (2015) ABCB1, ABCC2, SCN1A, SCN2A, GABRA1 gene

polymorphisms and drug resistant epilepsy in the Chinese Han population. Pharmazie 70(6):416–420
18. Rogawski MA, Loscher W (2004) The neurobiology of antiepileptic drugs. Nat Rev Neurosci 5(7):553–564
19. Kumari R, Lakhan R, Kalita J, Misra UK, Mittal B (2010) Association of alpha subunit of GABAA receptor subtype gene polymorphisms with epilepsy susceptibility and drug resistance in north Indian population. Seizure 19(4):237–241
20. Haerian BS, Baum L, Kwan P, Cherny SS, Shin JG, Kim SE, Han BG, Tan HJ, Raymond AA, Tan CT, Mohamed Z (2016) Contribution of GABRG2 polymorphisms to risk of epilepsy and febrile seizure: a multicenter cohort study and meta-analysis. Mol Neurobiol 53(8):5457–5467
21. Balan S, Sathyan S, Radha SK, Joseph V, Radhakrishnan K, Banerjee M (2013) GABRG2, rs211037 is associated with epilepsy susceptibility, but not with antiepileptic drug resistance and febrile seizures. Pharmacogenet Genomics 23(11):605–610
22. Loscher W (2002) Basic pharmacology of valproate: a review after 35 years of clinical use for the treatment of epilepsy. CNS Drugs 16(10):669–694
23. Tang L, Lu X, Tao Y, Zheng J, Zhao P, Li K, Li L (2014) SCN1A rs3812718 polymorphism and susceptibility to epilepsy with febrile seizures: a meta-analysis. Gene 533(1):26–31
24. Li X, Zhang J, Wu X, Yan H, Zhang Y, He RH, Tang YJ, He YJ, Tan D, Mao XY, Yin JY, Liu ZQ, Zhou HH, Liu J (2016) Polymorphisms of ABAT, SCN2A and ALDH5A1 may affect valproic acid responses in the treatment of epilepsy in Chinese. Pharmacogenomics 17(18):2007–2014
25. Kwan P, Poon WS, Ng HK, Kang DE, Wong V, Ng PW, Lui CH, Sin NC, Wong KS, Baum L (2008) Multidrug resistance in epilepsy and polymorphisms in the voltage-gated sodium channel genes SCN1A, SCN2A, and SCN3A: correlation among phenotype, genotype, and mRNA expression. Pharmacogenet Genomics 18(11): 989–998
26. Haerian BS, Baum L, Kwan P, Tan HJ, Raymond AA, Mohamed Z (2013) SCN1A, SCN2A and SCN3A gene polymorphisms and responsiveness to antiepileptic drugs: a multicenter cohort study and meta-analysis. Pharmacogenomics 14(10):1153–1166
27. DeVane CL (2003) Pharmacokinetics, drug interactions, and tolerability of valproate. Psychopharmacol Bull 37(Suppl 2):25–42
28. Marahatta A, Bhandary B, Jeong SK, Kim HR, Chae HJ (2014) Soybean greatly reduces valproic acid plasma concentrations: a food-drug interaction study. Sci Rep 4:4362
29. Golmohammadi R, Pejhan A, Azhdari-Zarmehri H, Mohammad-Zadeh M (2013) The role of ethanol on the anticonvulsant effect of valproic acid and cortical microvascular changes after epileptogenesis in mice. Neurol Sci 34(7):1125–1131
30. Feng W, Mei S, Zhu L, Yu Y, Yang W, Gao B, Wu X, Zhao Z, Fang F (2018) Effects of UGT2B7, SCN1A and CYP3A4 on the therapeutic response of sodium valproate treatment in children with generalized seizures. Seizure 58:96–100
31. Fisher RS, Acevedo C, Arzimanoglou A, Bogacz A, Cross JH, Elger CE, Engel J Jr, Forsgren L, French JA, Glynn M, Hesdorffer DC, Lee BI, Mathern GW, Moshe SL, Perucca E, Scheffer IE, Tomson T, Watanabe M, Wiebe S (2014) ILAE official report: a practical clinical definition of epilepsy. Epilepsia 55(4): 475–482
32. Berg AT, Berkovic SF, Brodie MJ, Buchhalter J, Cross JH, van Emde Boas W, Engel J, French J, Glauser TA, Mathern GW, Moshe SL, Nordli D, Plouin P, Scheffer IE (2010) Revised terminology and concepts for organization of seizures and epilepsies: report of the ILAE commission on classification and terminology, 2005-2009. Epilepsia 51(4):676–685
33. Meng H, Guo G, Ren J, Zhou H, Ge Y, Guo Y (2011) Effects of ABCB1 polymorphisms on plasma carbamazepine concentrations and pharmacoresistance in Chinese patients with epilepsy. Epilepsy Behav 21(1):27–30
34. Ellis JA, Ong B (2017) The MassARRAY(R) system for targeted SNP genotyping. Methods Mol Biol 1492:77–94
35. Clarke GM, Anderson CA, Pettersson FH, Cardon LR, Morris AP, Zondervan KT (2011) Basic statistical analysis in genetic case-control studies. Nat Protoc 6(2):121–133
36. Pedley TA, Hirano M (2003) Is refractory epilepsy due to genetically determined resistance to antiepileptic drugs? N Engl J Med 348(15):1480–1482
37. Dombrowski SM, Desai SY, Marroni M, Cucullo L, Goodrich K, Bingaman W, Mayberg MR, Bengez L, Janigro D (2001) Overexpression of multiple drug resistance genes in endothelial cells from patients with refractory epilepsy. Epilepsia 42(12): 1501–1506
38. Kwan P, Sills GJ, Butler E, Gant TW, Meldrum BS, Brodie MJ (2002) Regional expression of multidrug resistance genes in genetically epilepsy-prone rat brain after a single audiogenic seizure. Epilepsia 43(11):1318–1323
39. Gitai LL, de Almeida DH, Born JP, Gameleira FT, de Andrade TG, Machado LC, Gitai DL (2012) Lack of association between rs211037 of the GABRG2 gene and juvenile myoclonic epilepsy in Brazilian population. Neurol India 60(6):585–588
40. Dixit AB, Banerjee J, Ansari A, Tripathi M, Chandra SP (2016) Mutations in GABRG2 receptor gene are not a major factor in the pathogenesis of mesial temporal lobe epilepsy in Indian population. Ann Indian Acad Neurol 19(2):236–241
41. Rai V, Kumar P (2018) Methylenetetrahydrofolate reductase C677T polymorphism and susceptibility to epilepsy. Neurol Sci 39:2033–2041