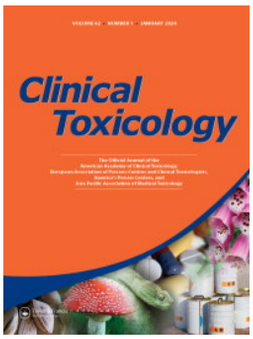

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CLINICAL RESEARCH

Sex differences in the susceptibility to valproic acid-associated liver injury in epileptic patients

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ABSTRACT

Background: Valproic acid has been widely used as an antiepileptic drug for several decades. Long-term valproic acid treatment is usually accompanied by liver injury. Although both men and women are susceptible to valproic acid-associated liver injury, hepatotoxicity differs between the sexes. However, the mechanisms underlying sex differences in valproic acid-associated liver injury remain unclear.

Methods: To explore potential risk factors for the susceptibility to valproic acid-associated liver injury, 231 pediatric patients with epilepsy (119 males, 112 females) were enrolled for laboratory and genetic analysis.

Results: Heterozygous genotype of *catalase* C-262T ($P = 0.045$) and the concentrations of glutathione ($P = 0.002$) and thiobarbituric acid-reactive substances ($P = 0.011$) were associated with the sex-specific susceptibility to valproic acid-associated liver injury. Meanwhile, logistic regression analysis revealed that carriers of heterozygous genotype of *catalase* C-262T ($P = 0.010$, odds ratio: 4.163; 95 percent confidence interval 1.400 – 7.378), glutathione concentration ($P = 0.001$, odds ratio: 2.421; 95 percent confidence interval 2.262 – 2.591) and male patients ($P = 0.005$, odds ratio: 1.344; 95% confidence interval 0.782 – 2.309) had a higher risk for valproic acid-associated liver injury.

Discussion: The mechanism underlying valproic acid-induced hepatotoxicity remains unclear. Additionally, factors that may contribute to the observed differences in the incidence of hepatotoxicity between males and females have yet to be defined. This study identifies several genetic factors that may predispose patients to valproic acid-associated hepatotoxicity.

Limitations: This relatively small sample size of children with one ethnicity some of whom were taking other antiepileptics that are potentially hepatotoxic.

Conclusion: *Catalase* C-262T genotype, glutathione concentration and gender (male) are potential risk factors for the susceptibility to valproic acid-associated liver injury.

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Valproic acid; sex differences; glutathione; *catalase* C-262T; liver injury

Introduction

Epilepsy is a common chronic central nervous system disorder characterized by the recurrence of unprovoked seizures. Owing to its chronic characteristics, long-term drug therapy is usually required for epileptic patients. Valproic acid is a broad-spectrum antiepileptic drug prescribed predominantly for epilepsy and psychiatric disorders [1]. Although valproic acid is effective and generally tolerated, long-term treatment can be accompanied by adverse drug reactions, such as hepatotoxicity, obesity, tremors and gut bacterial dysbiosis [2–5]. Among the adverse drug reactions caused by valproic acid, liver injury is one of the most common features. Although the underlying mechanisms of valproic-induced liver injury are not fully understood, oxidative stress has been postulated to be involved [6].

Oxidative stress happens when the balance between oxidants and antioxidants is disrupted, leading to excessive accumulation of reactive oxygen species [7]. Accordingly, antioxidant systems that consist of non-enzymatic antioxidants (e.g., glutathione, estradiol and carotenoid) and enzymatic antioxidants (e.g., glutathione S-transferases, glutathione peroxidase, catalase, and superoxide dismutase) are recruited to neutralize extra reactive oxygen species [8]. On the one hand, molecular antioxidants (especially glutathione) directly protect against valproic acid-induced oxidative stress, lipid peroxidation, and hepatotoxicity [9,10]. As such, mutations in genes encoding antioxidant enzymes may lead to the reduction to eliminate reactive oxygen species [2,11]. For example, the *glutathione peroxidase 1* variant (C-198T, Pro200Leu, rs1050450) is correlated with the susceptibility to idiosyncratic liver injury [12,13]. Similarly, patients with *catalase* C-262T (rs1001179) heterozygous genotype have a higher risk of valproic acid-induced liver dysfunction [14]. In addition, glutathione S-transferase mu 1 or glutathione S-transferase theta 1 null genotypes are believed to be related to carbamazepine or troglitazone-induced hepatotoxicity [15,16]. Moreover, the polymorphism of *superoxide dismutase 2* (Val16Ala, rs4880) contributes to

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valproic acid-induced elevation of gamma-glutamyl transferase [17]. However, the possible connection between antioxidant system and valproic acid-induced liver injury has not been well studied.

Individual susceptibility to drug metabolism and pharmacokinetics may present major changes in clinical pharmacology and toxicology [18]. Indeed, a previous study showed that women had a lower valproic acid hepatic output and a higher reabsorbed fraction than men, suggesting that valproic acid displayed sex-related pharmacokinetic differences [19]. However, the underlying mechanism for sex differences in valproic acid-induced liver injury is still unclear. Genetic polymorphisms of antioxidant enzymes and molecular antioxidant concentrations may be a new approach to demonstrate the correlation between sex differences and valproic acid-induced liver injury. Hence, this study was designed to systematically investigate the associations of genetic polymorphisms and oxidative stress parameters with valproic acid-associated liver injury in male and female patients with epilepsy.

Materials and methods

Patients

This study included 231 age-matched pediatric patients from the Han Chinese population. All patients were recruited (from August 2022 to March 2023) at Yuhuangding Hospital and diagnosed with epilepsy according to the etiological classification of epilepsy [20]. Patients underwent valproic acid-based therapy for more than three months to ensure a steady-state valproic acid concentration. The sex-specific demographic characteristics (such as age, body-mass index and liver function) of each patient were recorded. Male and female patients were divided into those with liver injury (at least one indicator exceeding the upper limit of normal) group and control (all liver function indicators did not exceed the upper limit of normal) group according to their liver function tests. Meanwhile, patients with pre-existing liver injury or other potential causes of liver disease (such as hepatitis B, HIV-positive or alcohol use disorder) were excluded from this study.

Informed consent was obtained from patients or their legal guardians. This study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Qingdao University.

Blood collection and laboratory assays

After fasting overnight for approximately 10 h (minimal fasting period of 6 h for children up to the age of 3 years), venous blood of each patient was collected using sterile clot activator tubes. Blood samples were centrifuged at 3,500 rpm for 5 min and then analyzed within 30 min or stored at −80 °C for later analysis. Liver function tests, such as alanine aminotransferase (ALT) activity, aspartate aminotransferase (AST) activity, alkaline phosphatase (ALP) activity, gamma-glutamyl transferase (GGT) activity and total bilirubin concentration were determined by an automatic biochemistry analyzer (Au5800, Beckman Coulter, USA). The concentration of glutathione was measured according to published methodology [21]. Serum superoxide dismutase activity and thiobarbituric acid-reactive substances concentrations were determined by Beckman Coulter ACCESS® (Brea, CA, USA) and suitable kits according to the manufacturer's instructions.

Quantification of valproic acid concentrations

Peripheral blood samples were collected using disodium ethylenediaminetetraacetic acid (EDTA) tubes. A total of 5 mL of whole blood was drawn from each patient for DNA extraction. To measure steady-state valproic acid concentrations, blood samples were collected just before the last valproic acid administration. The valproic acid concentration was quantified using an automatic fluorescence immunoassay system (Abbott, Chicago, USA) as described previously [14].

Genotyping procedures

Whole blood was collected from each subject and genomic DNA was extracted using a DNA Extraction Kit (OMEGA, Norcross, USA). Genetic polymorphisms of *glutathione peroxidase 1* C-198T, *catalase* C-262T, *superoxide dismutase 2* Val16Ala and *glutathione S-transferase mu 1/theta 1* were determined according to the protocols described previously [22]. Primer sequences used for genotyping are listed in Supplementary Table 1.

Statistical analysis

Statistical analyses were performed using SPSS (version 20.0; IBM, USA). Data are presented as mean ± standard deviation. The demographic characteristics of the patients were determined using the Student's *t* test. Statistical significance of genotype frequencies was determined using the chi-square test. Logistic regression was used to evaluate the risks of demographic characteristics and genotypes for valproic acid-associated liver dysfunction. A *P* value less than 0.05 represents statistically significant.

Results

Sex differences in demographic characteristics in epileptic patients

A total of 231 pediatric patients (119 males, 112 females) with valproic acid-based therapy were recruited. The concomitant drugs used for each patient were also recorded and summarized in Supplementary Table 2. Specifically, clonazepam (10 patients), topiramate (five patients), carbamazepine (14 patients), lamotrigine (15 patients), oxcarbazepine (three patients) and levetiracetam (nine patients) were combined with valproic acid. No significant differences were observed in concomitant drugs between male and female patients ($P > 0.05$, Supplementary Table 2).

Table 1. Demographic characteristics and biochemical indicators of patients.

	Liver injury group ($n = 90$)			Control group ($n = 141$)		
Demographic characteristics	Male	Female	*P* value	Male	Female	*P* value
Number of patients	53 (58.9%)	37 (41.1%)	–	66 (46.8%)	75 (53.2%)	–
Age (years)	6.81 ± 6.44	6.66 ± 8.70	0.922	6.69 ± 5.17	6.06 ± 4.58	0.447
Height (cm)	116.33 ± 41.50	101.01 ± 33.47	0.056	115.26 ± 31.39	106.73 ± 33.61	0.121
Body weight (kg)	29.22 ± 21.74	22.38 ± 19.25	0.127	25.27 ± 13.37	22.77 ± 12.55	0.257
Body mass index (kg/m^2)	19.33 ± 5.04	19.07 ± 4.72	0.804	18.74 ± 7.64	19.17 ± 7.64	0.741
Total bilirubin concentration (μmol/L) [mg/dL]	8.01 ± 5.68 [0.47 ± 0.33]	7.64 ± 3.57 [0.45 ± 0.21]	0.726	6.63 ± 2.54 [0.39 ± 0.15]	6.51 ± 2.82 [0.38 ± 0.16]	0.822 0.822
Gamma-glutamyl transferase activity (U/L)	27.49 ± 19.16	24.13 ± 12.91	0.371	18.94 ± 14.11	16.06 ± 5.69	0.252
Alkaline phosphatase activity (U/L)	202.78 ± 125.52	216.42 ± 59.09	0.576	208.10 ± 114.76	210.33 ± 47.89	0.913
Alanine aminotransferase activity (U/L)	60.23 ± 45.79	42.34 ± 23.79	**0.003**	12.56 ± 5.61	14.12 ± 9.29	0.344
Aspartate aminotransferase activity (U/L)	57.34 ± 31.48	44.14 ± 17.87	**0.013**	25.95 ± 9.94	26.49 ± 7.39	0.785

Data are presented as mean ± standard deviation. The bolded data indicate $P < 0.05$.
Statistical significance was determined by Student's *t*-test for independent samples.

Table 2. Comparisons of valproic acid doses, concentrations and oxidative parameters in patients.

	Liver injury group ($n = 90$)			Control group ($n = 141$)		
Items	Male	Female	*P* value	Male	Female	*P* value
Number of patients	53 (58.9%)	37 (41.1%)	–	66 (46.8%)	75 (53.2%)	–
Valproic acid concentration (mg/L)	57.14 ± 29.38	59.95 ± 25.54	0.638	62.98 ± 26.66	61.60 ± 26.37	0.759
Valproic acid daily doses (mg/kg)	18.37 ± 8.06	20.27 ± 7.62	0.255	20.29 ± 8.46	18.59 ± 7.85	0.222
Adjusted valproic acid concentration [(mg/L)/(mg/kg)]	3.22 ± 1.14	3.10 ± 1.18	0.637	3.45 ± 1.61	4.21 ± 2.63	0.264
Glutathione concentration (μmol/L)	32.85 ± 4.32	37.25 ± 3.22	**0.002**	47.93 ± 4.80	48.08 ± 3.61	0.845
Superoxide dismutase activity (U/mL)	3.76 ± 2.40	3.34 ± 1.43	0.578	3.11 ± 1.16	3.09 ± 1.25	0.929
Thiobarbituric acid-reactive substances concentrations (μmol/L)	2.47 ± 0.51	2.07 ± 0.17	**0.011**	1.96 ± 0.28	1.97 ± 0.18	0.710

Data are presented as mean ± standard deviation.
Statistical significance was determined by Student's *t*-test for independent samples.

In this study, all patients were divided into the liver injury ($n = 90$) and control ($n = 141$) groups. A summary of the sex-specific demographic characteristics is presented in Table 1. As shown in Table 1, the percentage of male patients with liver injury was higher than that of female patients (58.9% versus 41.1%) in the liver injury group. Importantly, the activities of ALT and AST in male patients were approximately 42% and 30% greater than those in female patients ($P < 0.05$) respectively, suggesting male patients may more susceptible to valproic acid-associated liver injury. In the control group, liver function did not differ between male and female patients. However, the underlying mechanisms for sex differences in valproic acid-associated liver injury is still unclear.

Comparisons of valproic acid concentrations and oxidative stress parameters between male and female patients

To explore the possible mechanisms for sex differences in valproic acid-associated liver injury, valproic acid doses/concentrations in male and female patients were analyzed. As shown in Table 2, valproic acid dose, valproic acid concentration and dose-adjusted valproic acid concentration did not differ between the liver injury and control patients (even after modified by sex), suggesting that valproic acid dose and concentrations may not alter valproic acid-associated liver injury.

Oxidative stress is well known to be involved in the progression of valproic acid-associated hepatotoxicity. Hence, in this study, we examined oxidative stress parameters (glutathione, superoxide dismutase and thiobarbituric acid-reactive substances) in patients. As presented in Table 2, a significantly lower glutathione and a higher thiobarbituric acid-reactive substances concentrations were observed in patients with liver injury than that in control patients ($P < 0.05$). Critically, the concentrations of glutathione and thiobarbituric acid-reactive substances in male patients are approximately 11.3% lower and 11.9% higher than that in female patients ($P < 0.05$). These results indicated that oxidative stress may be responsible for the sex-specific susceptibility to valproic acid-associated liver injury.

Sex differences in genetic polymorphisms and valproic acid-associated liver injury

To further investigate the relationship between sex differences and valproic acid-induced liver injury, single nucleotide polymorphisms in the four antioxidant enzymes were determined in the liver injury patients and control patients. The sex-related frequency of each genotype was consistent with the Hardy-Weinberg equilibrium. As shown in Table 3, 16 carriers (17.8%) of heterozygous genotype of *CAT* C-262T were detected in patients with liver injury ($n = 90$), while only six carriers (4.3%) of heterozygous genotype of *CAT* C-262T in the control group ($n = 141$). Importantly, the heterozygous frequency of *CAT* C-262T was significantly higher in male patients than that in female patients (24.6% versus 8.1%, $P = 0.045$) in the liver injury group. Moreover, carriers of the heterozygous genotype of *CAT* C-262T (CT) had higher ALT and AST activities than the carriers of the CC genotype of *CAT* C-262T ($P = 0.004$ and 0.011, respectively. Supplementary Table 3). However, there was no significant

difference in *CAT* C-262T genotype (4.5% versus 4.0%, $P = 0.873$) between the male and female patients in the control group. These results suggest that *CAT* C-262T may be associated with the sex-specific susceptibility to valproic acid-associated liver injury in male patients with epilepsy. Furthermore, although no differences in *glutathione S-transferase mu 1/theta 1* null genotypes, *superoxide dismutase 2* Val16Ala and *glutathione peroxidase 1* C-198T polymorphisms were detected between the liver injury and control groups ($P > 0.05$), carriers of heterozygous genotype of *glutathione peroxidase 1* C-198T (CT) had a higher ALT and AST activities than patients with the CC genotype of *glutathione peroxidase 1* C-198T ($P = 0.029$ and 0.003, respectively. Supplementary Table 3).

Sex differences in the risk factors for valproic acid-associated liver injury

To further clarify the sex-specific influence of demographic characteristics and genetic polymorphisms on valproic acid-associated liver injury, logistic regression analysis was performed to identify the risk factors for valproic acid-associated liver injury.

As shown in Table 4, the results indicated that the T allele in *catalase* C-262T variants ($P = 0.010$), glutathione concentration ($P = 0.001$) and gender (male, $P = 0.005$) were risk factors for VPA-associated liver injury. The odds ratios (ORs) of *CAT* C-262T heterozygous genotypes, glutathione concentration and gender (male) were 4.163 (95% confidence interval 1.400 – 7.378), 2.421 (95% confidence interval 2.262 – 2.591) and 1.344 (95% confidence interval 0.782 – 2.309) respectively. Moreover, there were no significant differences in age, body-mass index, valproic acid dose and concentration, *glutathione S-transferase mu 1/theta 1* and *superoxide dismutase 2* Val16Ala or *glutathione peroxidase 1* C-198T genotypes ($P > 0.05$). Based on these findings, *catalase* C-262T heterozygous genotypes, glutathione concentrations and gender (male) are risk factors for the susceptibility to valproic acid-associated liver injury.

Discussion

Valproic acid is a widely prescribed antiepileptic drug for the treatment of epilepsy and bipolar disorders. Long-term valproic acid treatment is associated with metabolic dysfunction and liver diseases [2]. However, the mechanism underlying VPA-induced hepatotoxicity remains unclear. For a long time, oxidative stress has been identified as a risk factor in the pathogenesis of liver disease. This study was designed to explore potential risk factors for the susceptibility to valproic acid-induced liver injury, and investigate risk factors in epileptic patients. In this study, we first demonstrated *catalase* C-262T heterozygous genotypes, glutathione concentration

Table 3. Comparisons of *glutathione S-transferases*, *superoxide dismutase 2*, *glutathione peroxidase 1*, and *catalase* genotypes frequencies between male and female patients.

	Liver injury group				Control group			
Genotypes	Male $n = 53$	Female $n = 37$	Odds ratio (95% confidence interval)	*P* value	Male $n = 66$	Female $n = 75$	Odds ratio (95% confidence interval)	*P* value
Glutathione S-transferase mu 1/ theta 1								
M1+/T1+	17 (32.1%)	10 (27.0%)	Reference		26 (39.4%)	25 (33.3%)	Reference	
M1+/T1-	9 (16.9%)	9 (24.3%)	1.350 (0.688 – 2.651)	0.388	12 (18.2%)	18 (24.0%)	1.224 (0.817 – 1.834)	0.339
M1-/T1+	9 (17.0%)	7 (18.9%)	1.181 (0.562 – 2.481)	0.663	14 (21.2%)	14 (18.7%)	1.020 (0.641 – 1.623)	0.934
M1-/T1-	18 (34.0%)	11 (29.8%)	1.024 (0.520 – 2.016)	0.945	14 (21.2%)	18 (24.0%)	1.148 (0.758 – 1.737)	0.521
Superoxide dismutase 2 Val16Ala								
TT	39 (73.6%)	26 (70.2%)	Reference		45 (68.2%)	51 (68.0%)	Reference	
TC + CC	13 + 1 (26.4%)	10 + 1 (29.8%)	1.100 (0.645 – 1.875)	0.730	20 + 1 (31.8%)	21 + 3 (32.0%)	1.004 (0.721 – 1.399)	0.982
Glutathione peroxidase 1 C-198T								
CC	43 (81.1%)	32 (86.4%)	Reference		59 (89.4%)	70 (93.3%)	Reference	
CT	10 (18.9%)	5 (13.6%)	1.488 (0.463 – 4.781)	0.502	7 (10.6%)	5 (6.7%)	1.685 (0.508 – 5.585)	0.390
Catalase C-262T								
CC	40 (75.4%)	34 (91.9%)	Reference		63 (95.5%)	72 (96.0%)	Reference	
CT	13 (24.6%)	3 (8.1%)	3.683 (0.968 – 14.011)	**0.045**	3 (4.5%)	3 (4.0%)	1.143 (0.415 – 2.119)	0.873

The bolded data indicate $P < 0.05$. Statistical significance was determined by the chi-square test.

Table 4. Logistic regression analysis of risk factors for valproic acid-associated liver injury.

Variables	Regression coefficient	*P* value	Exp (B)	95% Confident interval
Age	0.100	0.139	1.105	0.968 ~ 1.261
Body mass index	0.640	0.285	0.972	0.780 ~ 2.309
Gender (male)	0.295	**0.005**	1.344	0.782 ~ 2.309
Glutathione concentration	−0.884	**0.001**	2.421	2.262 ~ 2.591
Thiobarbituric acid-reactive substances concentrations	0.186	0.368	1.204	0.803 ~ 1.806
Glutathione S-transferase mu 1/ theta 1	0.282	0.363	1.325	0.723 ~ 2.430
Superoxide dismutase 2 Val16Ala	−0.146	0.626	0.864	0.481 ~ 1.554
Glutathione peroxidase 1 C-198T	0.495	0.421	1.641	0.717 ~ 3.756
Catalase C-262T	1.426	**0.010**	4.163	1.400 ~ 7.378
Valproic acid dose	−0.083	0.241	0.921	0.849 ~ 0.999
Valproic acid concentration	0.017	0.202	1.017	0.991 ~ 1.044
Adjusted valproic acid concentration	−0.436	0.085	0.647	0.394 ~ 1.062

The bolded data indicate $P < 0.05$.

and gender (male) are involved in the sex-specific susceptibility to valproic acid-associated liver injury.

Oxidative stress is a consequence of an imbalance in the production and elimination of reactive oxygen species [23]. Early studies demonstrated that reactive oxygen species plays a key role in the progression of valproic acid-associated hepatic diseases [10,24]. Meanwhile, there is a growing body of evidence that defense against reactive oxygen species is usually more efficient in females than that in males, leading to sex-biased initiation and progression of liver disease [25]. Therefore, a deficiency in antioxidant defenses could be a risk factor for sex differences in valproic acid-associated liver injury. In this study, we examined the genetic polymorphisms of antioxidant enzymes and concentrations of molecular antioxidants. The results showed that carriers of *catalase* C-262T heterozygous genotypes is associated with a higher risk of valproic acid-associated liver injury ($P = 0.010$, odds ratio: 4.163; 95% confidence interval 1.400 – 7.378), which is consistent with our previous study [14]. However, our research in 2019 did not focus on the sex differences in the prevalence of valproic acid-associated liver injury. The current study further demonstrated that *catalase* C-262T genotypes is associated with the sex-specific susceptibility (24.6% versus 8.1%, $P = 0.045$) to valproic acid-associated liver injury in patients with epilepsy. Moreover, although early studies indicated that *superoxide dismutase 2* Val16Ala, *glutathione peroxidase 1* C-198T polymorphism, *glutathione S-transferase mu 1* and *glutathione S-transferase theta 1* null genotypes are associated with valproic acid-induced hepatotoxicity [15–17], we did not observe any significant correlations between these three polymorphisms and valproic acid-induced liver injury, even after modifying by sex. Furthermore, the prevalence of *catalase* C-262T, *superoxide dismutase 2* Val16Ala and *glutathione peroxidase 1* C-198T mutant genotypes are approximately 9.5%, 34.6% and 11.7%, respectively. These results are similar to the prevalence reported in our previous study (7.9%, 29.5% and 13.4%, respectively) [14].

Glutathione, the most abundant antioxidant, is important for protecting the cells from oxidative stress, acting as a free radical scavenger and inhibitor of lipid peroxidation [9,26]. Critically, depletion of glutathione was confirmed to be associated with increased hepatic concentrations of lipid peroxides, activity of myeloperoxidase, and hepatotoxicity in valproic acid-treated rats [27]. In addition, our previous study demonstrated that glutathione significantly inhibits valproic acid-induced oxidative stress and attenuates valproic acid-induced hepatic steatosis [10]. Furthermore, glutathione also conjugates of active valproic acid metabolites (4-ene-valproic acid and 2,4-diene-valproic acid) for a "detoxification" in rats and humans [28,29]. However, to the best of our knowledge, the effect of glutathione on sex-specific susceptibility to valproic acid-induced liver dysfunction has rarely been investigated. In this study, male patients in liver injury group had a lower concentration of glutathione and a higher concentration of thiobarbituric acid-reactive substances than that in female patients and control patients. Meanwhile, logistic regression analysis also showed that glutathione was a risk factor for valproic acid-associated liver injury. These results suggesting that the glutathione concentration may contribute to the susceptibility to valproic acid-associated liver injury.

Finally, the limitations of this study should also be discussed. First, many antiepileptic drugs (such as valproic acid, carbamazepine, lamotrigine and topiramate) are associated with hepatotoxicity [30–32]. In this study, although patients underwent concomitant drug therapy only when they suffered a high frequency of recurrence of unprovoked seizures or according to the clinician's advice, the effects of concomitant drugs on liver injury cannot be completely excluded. In addition, all of the subjects were children with a similar ethnic background. However, our findings may explain (at least partly) the sex differences in the susceptibility to valproic acid-associated liver injury. Then, other factors (such as environmental conditions, daily living habits and diet) may also contribute to valproic acid-associated liver injury, which are very difficult to exclude from this cross-sectional study.

Conclusion

In conclusion, this study demonstrated that the *catalase* C-262T genotype, glutathione concentration and gender (male) may serve as risk factors for susceptibility to valproic acid-induced liver injury. We emphasize the importance of pre-identification of *catalase* C-262T genotype and glutathione concentration in patients receiving long-term valproic acid treatment (especially in male patients). This study may be helpful for the prevention and guidance of valproic acid-induced liver injury. Further research and clinical validation are warranted to validate these findings and explore their implications for personalized medicine in epilepsy treatment.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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