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Correlation of GSTP1 rs1695 and CAT rs769217 with elevated AST induced by valproate sodium in Chinese children with epilepsy

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Abstract: Valproate (VPA)-induced hepatotoxicity is a fatal adverse drug reaction， and children is a high-risk population. Our study aimed to explore whether key genetic polymorphisms of antioxidant pathway is associated with VPA-mediated AST elevation. We included 194 newly diagnosed epilepsy children (aged from 1 to 16 years old) and treated with VPA. These patients were divided into two groups: one group for AST is normal and another group is AST elevated. AST elevation occurred in 25.8% of patient treated with VPA. During VPA monotherapy, the maximum AST in patients of *GSTP1* rs1695 with AA genotype was significantly higher than carrying G alleles (36.50 ±14.89 vs 32.88±10.69, P=0.003). Patients with AG+GG genotype of GSTP1 rs1695 had a reduced risk of elevated AST (adjusted OR=0.37, 95% CI: 0.16-0.84, P=0.017). There is a significant difference in the maximum AST value of CAT rs769217 genotype (P=0.011, P= 0.045, respectively). Children with CAT rs769217 CT genotype or CT+TT genotype have a lower risk of elevated AST (adjusted OR=0.30, 95% CI: 0.13-0.68, P=0.004 and adjusted OR=0.41, 95% CI:0.20-0.82,P=0.012, respectively). Children who with *GSTP1* rs1695 G allele have a reduced risk of AST abnormalities. We conducted *CAT* rs769217 CC genotype is a risk factor for AST elevation in children.

Keywords: *GSTP1*, *PON1*, *CAT*, hepatotoxicity, epilepsy, valproate, children, AST.

INTRODUCTION

Valproate (VPA) is a classic antiepileptic drug. It is clinically used for monotherapy and multiple therapy of various types of epilepsy in children and adults. VPA is better tolerated than other antiepileptic drugs, but serious adverse reactions such as liver toxicity, pancreatitis, and teratogenicity limit its clinical application. Hepatotoxicity incidence in every 20,000 patients treated with VPA is less than 1% (Bryant and Dreifuss, 1996). Liver function abnormalities such as elevated transaminase are one of the most common adverse reactions of VPA(Nanau and Neuman, 2013). In up to 44% of patients, elevated transaminase may be related to chronic VPA use in the first few months of treatment(Lheureux *et al*., 2005). Dose-related liver injury is type I adverse reaction, which is usually temporary and manifests as mild to moderate increase in transaminase levels. Idiopathic hepatotoxicity belongs to type II adverse reactions. It is rare but often fatal, especially in children under 2 years old and who has diseases such as developmental delay or metabolic disorders (especially mitochondrial dysfunction), and simultaneous enzyme induction children on medication(Price *et al*., 2011). Children are at high risk of VPA-induced liver toxicity. An early study confirmed that the risk of VPA-induced hepatotoxicity is indeed higher for children under 2 years of age (Romoli *et al*., 2019). In recent years, studies have reported that patients with normal liver function will develop non-alcoholic fatty liver disease after using VPA, especially in adolescents(Farinelli *et al*., 2015). Therefore, VPA-induced hepatotoxicity should be highly valued in children. It is of great significance to find highly sensitive biomarkers that can predict VPA hepatotoxicity.

Formation of reactive metabolites of sodium valproate, inhibition of β-oxidation of fatty acids, excessive oxidative stress and genetic variation of certain enzymes such as glutathione sulfhydryl transferases (*GSTs*)、superoxide dismutase 2 (*SOD2)*、Uridine diphospho-glucuronosyl transferases *(UGTs)* and *CYP* genes are related to the liver toxicity of sodium valproate(Romoli *et al*., 2019). In addition, carnitine supplementation and taking antioxidants have been shown to be active treatment strategies for VPA-induced liver toxicity (Guo *et al*., 2019).

The main mechanisms of VPA-induced liver toxicity are the toxic metabolite theory and the oxidative stress theory. The active metabolites of VPA, 4-allyproic acid and 2, 4-diallyproic acid, have liver toxicity and can inhibit mitochondrial β-oxidation(Begriche *et al*., 2011). The elimination of VPA-induced liver toxicity often requires

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the participation of reduced glutathione (GSH) and antioxidant enzymes. The main antioxidant enzymes *in vivo* are catalase (CAT), paraoxonase 1 (PON1) and so on. Animal experiments have shown that the activity of GST plays an important role in chemically induced hepatotoxicity. Decreasing the expression of GST and depleting GSH can increase the risk of hepatotoxicity, and increasing the expression of GST will have an anti-hepatotoxic effect (Gum *et al*., 2007, Chen *et al*., 2020, Zhao *et al*., 2002). Most of the studies are about *GSTT1, GSTM1* and *GSTP1*. Deletion of *GSTM1* gene is associated with hepatotoxicity mediated by anti-tuberculosis drugs (Huang *et al*., 2007, Li *et al*., 2013a, Roy *et al*., 2001) . The simultaneous deletion of *GSTT1* and *GSTM1* genes will increase the liver toxicity of tacrine, troglitazone, carbamazepine, anti-tuberculosis, anti-inflammatory drugs, nonsteroidal anti-inflammatory drug and amoxicillin-clavulanate potassium (Simon *et al*., 2000, Watanabe *et al*., 2003, Lucena *et al*., 2008). *GSTM1-* and *GSTM1-/GSTT1-* are risk factors for VPA-mediated increase of γ-glutaminase(Fukushima *et al*., 2008). The π isoenzyme of GSTs encoded by *GSTP1* is an important phase II metabolic enzyme in the human body. GSTs π is a major role in regulating the MAP kinase pathway through protein interaction(Townsend and Tew, 2003). The *GSTP1* rs1695 mutation is a change of I105V on exon 5 of the *GSTP1* gene, which causes the 105 amino acid to change from isoleucine (Ile) to valine (Val). Isoleucine is one of the amino acids in the binding site of the hydrophobic substrate of *GSTP1*. This site mutation will change the volume and hydrophobicity of amino acids, reduce the thermal stability of the enzyme, then cause changes in the affinity of the enzyme to the substrate and its specific catalytic activity. Missense mutations of *GSTP1-1* affect enzyme activity(Johansson *et al*., 1998). Some studies have indicated that *GSTP1* exon 5 polymorphism increased hepatocellular carcinogenesis (HCC) risks (Chen *et al*., 2010, Li *et al*., 2012), another clinical research in Thai male patients shows no association(Sophonnithiprasert *et al*., 2020). There are no statistical significance between the GSTP1 Ile105 Val polymorphism and risk of anti-tuberculosis mediated liver injury in Tuberculosis patients, but Shouquan Wu *et al*. found that the risk of anti-tuberculosis induced hepatotoxicity in patients with GSTP1 rs1695 allele A is higher (He *et al*., 2015, Wu *et al*., 2016). These researches showed that *GSTP1* may affect liver function, but GSTP1 is controversial for drug-mediated hepatotoxicity. Current studies on *GSTP1* and VPA-mediated AST elevation are still limited.

The paraoxonase (PON) gene family consists of *PON1*, *PON2*, and *PON3*. At present, the most researched is *PON1*, which is a type of calcium-dependent esterase. *PON1* can hydrolyze organophosphorus, prevent oxidative stress and anti-inflammatory, and play an major role in the detoxification process of organophosphorus neurotoxic agents. The missense mutation at position rs662 of *PON1* changed the amino acid at position 192 of the protein sequence from glutamine to arginine. And the gene polymorphism of *PON1* is also closely related to diseases such as atherosclerosis, coronary heart disease and type 2 diabetes(Précourt *et al*., 2011) . The missense mutation of *PON1* rs662 will affect the activity of the enzyme. The hydrolytic activity of the *PON1* enzyme containing arginine is significantly higher than that of the enzyme containing glutamine(Humbert *et al*., 1993). A study suggested that the PON1/Aryl activities decreased in children after VPA therapy, which may be related to liver dysfunction and VPA production of free radicals, but the direct effect of drugs on enzymes cannot be ruled out(Karikas *et al*., 2009). At present, there are still no studies about PON1 rs 662 and VPA mediated liver toxicity.

Catalase (CAT) is an important antioxidant enzyme in the biotransformation pathway of ROS, which can catalyze the conversion of H202 into water and prevent cell damage and apoptosis caused by ROS. The function of the *CAT* enzyme is determined by the protein encoded by the *CAT* gene. Mutations in the *CAT* gene may cause related proteins abnormal expression or dysfunction. *CAT* rs769217 C>T is a synonymous mutation in exon 9 of the *CAT* gene, which will affect DNA transcription, and finally influence *CAT* enzyme activity(Saify *et al*., 2016). The most common polymorphisms that affect *CAT* gene transcription and influence the activity of *CAT* are -262C/T, -844G/A or -844C/T in the promoter region(Nandi *et al*., 2019). Linfeng Ma *et al*. found that *CAT* (C-262T, rs1001179) polymorphism is a risk factor for VPA-induced abnormal liver function in Chinese patients with epilepsy(Ma *et al*., 2019). A significant association of bisphenol A with abnormal liver function was observed in participants with CAT rs769217(Kim *et al*., 2016). But the correlation on CAT rs769217 and VPA induced AST elevation is still needs further study.

In this study, we collected clinical data and blood samples of children with epilepsy who were treated with VPA, monitored the steady-state blood trough concentration of VPA and regularly carried out follow-up the patients. Based on the theory of "toxic metabolites" and "oxidative stress", the polymorphic sites of the key enzyme genes *GSTP1*, *PON1* and *CAT* in the oxidative stress pathway were selected. We analyzed the correlation between genetic polymorphisms of these genes and VPA-mediated AST abnormalities, aiming to predict VPA liver toxicity based on SNPs variation, so as to realize the individualized of VPA treatment and provide a reference for clinical rational drugs use.

MATERIALS AND METHODS

Patients

This study included 194 Chinese children with newly-

diagnosed epilepsy (1-16 years old) who were treated at Xiangya Hospital of Central South University from January 2015 to December 2017. Patients' demographic and clinical data were collected at their first visit, such as name, gender, age, body weight, height, medical and neurological history, family history of epilepsy, frequency and duration of seizures, and epilepsy treatment regimen. The criteria of inclusion in our research is Chinese Han nationality and age from 1 to 16 years old, patients with newly-onset epilepsy diagnosed by clinical and EEG, first use of sodium valproate monotherapy for anti-epileptic treatment. Participants are required to regularly participate in the monitoring of steady-state trough concentration of VPA and the tests of liver function and blood routine. Patients were excluded if the liver and kidney function was abnormal before medication or suffered from infectious diseases, serious organic diseases or any progressive diseases. Patients who concomitant use of other anti-epileptic drugs and some drugs that affect liver function were also excluded. Patients with poor medication adherence were excluded.

All patients were informed of the research purpose, research methods and significance before enrollment, and signed an informed consent form. Blood routine, liver and kidney function, blood ammonia and other biochemical indicators were tested by Laboratory Medicine Department, Xiangya Hospital, Central South University. E.Z.N.A. ® Blood DNA Midi Kit. With informed consent, the selected participants collected 2 mL of fasting venous blood and placed it in an EDTA anticoagulation tube for DNA extraction. The plan of this subject was approved by the ethics committee of Xiangya Hospital of Central South University (Lot No. 201608611).

Evaluation of adverse reactions

Study on VPA-induced AST abnormalities: Follow up the patients included in the study and record the steady-state blood trough concentration of VPA, liver and kidney function examination value, and blood routine. Abnormal liver function is defined as the maximum value of liver function indexes ALT and AST higher than the upper limit of the normal range of ALT and AST during the treatment of VPA monotherapy. The upper limit of normal AST for men is 40 U/L while the upper limit of normal AST for women is 35 U/L. VPA monotherapy patients were divided into two groups according to their AST values: patients whose AST values were within the normal range were included in the normal group, and those with above the upper limit of the normal range were included in the abnormal group.

DNA extraction and Genotyping

After the subjects took VPA for at least 1 month, draw 2 mL of peripheral venous blood with a steady-state trough concentration before taking the medicine on an empty stomach, and place it in an EDTA anticoagulation tube. The upper plasma is used for VPA Plasma concentration detection, and the lower blood cells are stored at 4°C for DNA extraction. Plasma concentration detected by Agilent, USA 7890A gas chromatograph. (Omega) was used for DNA extraction, and the DNA concentration, purity and degree of degradation were detected by a protein analyzer.

Genotyping uses the SNPscan typing method. The SNPs information of the antioxidant pathway genes included in this study is depicted in table 1.

In order to verify the accuracy of genotyping, the study set up the genotyping of random duplicate samples as an internal control, that is randomly selecting some patients' DNA for two genotyping and comparing the consistency of genotyping between the two duplicate samples.

STATISTICAL ANALYSIS

Statistical analyses were performed using the SPSS software (IBM SPSS Statistics 18). The sample size was calculated based on the number of independent variables in the logistic regression model. The number of subjects must be at least 10 times the number of included independent variables, as per the sample size inclusion principle for logistic regression analysis. The Hardy-Weinberg equilibrium analysis was performed with Pearson$\chi 2$ test to ensure that the selected patients are representative of the population and the reliability of the results. Continuous variables use t test or nonparametric test Mann Whitney U test, categorical variables use chi-square test. Each gene locus and the risk of abnormal liver function during VPA treatment was analyzed by unconditional logistic analysis method, balancing the confounding factors such as age, gender, dose per weight, then obtain odds ratio (OR) and 95% confidence interval (95% CI). $P<0.05$ has statistically significant.

RESULTS

The results of genetic polymorphisms and VPA-induced liver toxicity

A total of 194 children with epilepsy undergoing VPA monotherapy were enrolled in this study. During the treatment, 50 cases developed AST abnormalities. The incidence of abnormal adverse reactions caused by VPA-induced AST abnormity was 25.8%. The basic demographic data and clinical data of the AST normal group and abnormal group during VPA treatment are shown in table 2. Among these patients, 144 patients were classified into the AST normal group and 50 were in the AST abnormal group. In the AST normal group, 42 were males and 102 were females, aged 6±4 years old. And there were 50 cases in the abnormal AST group, including 17 males and 33 females, aged 4±3 years.

Table 1: Information for key SNPs of antioxidant pathway included studies

Gene	SNP	Chr	Region	Allele	Variant	FS score	MAF $_{CHB}$
GSTP1	rs1695	11	exon5	A>G	p.I105V	0.365	0.19
PON1	rs662	7	exon6	T>C	p.Q192R	0.396	0.62
CAT	rs769217	11	exon9	C>T	Synonymous variant	0.280	0.53

Table 2: Demographic data and clinical characteristics of the patients at the time when the AST reached the maximum value for each patient during the study period

Parameters	Total (n=194)	Normal (n=144)	Elevated (n=50)	*P*
Men/Women	59/135	42/102	17/33	0.522
Age(years)	6±4	6±4	4±3	0.079
Body weight(kg)	22.9±12.9	24.5±13.7	18.0±8.8	**0.008**
VPA dose(mg/day)	394±212	406±197	361±250	0.770
VPA dose(mg/kg/day)	19±12	19±13	19±8	0.885
VPA concentration (μg/mL)	51.43±24.11	50.59±24.03	53.85±24.41	0.788

NOTE: VPA: valproic acid; ALT: alanine aminotransferase; AST: aspartate aminotransferase; Continuous variables use t test or nonparametric test Mann Whitney U test, categorical variables use chi-square test. The differences were considered to be significant at $p < 0.05$.

Table 3: The distribution of the genotype and allele in patients

Gene	SNP	Genotype	N	Identified Frequency (%)	Allele	Allele frequency (%)	P_{hwe}
GSTP1	rs1695	AA	128	66.0	A	82.0	
		AG	62	31.9	G	18.0	0.26
		GG	4	2.1			
PON1	rs662	CC	86	44.3	C	64.9	
		CT	80	41.2	T	35.1	0.19
		TT	28	14.5			
CAT	rs769217	CC	68	35.1	C	56.4	
		CT	83	42.8	T	43.6	0.07
		TT	43	22.2			

Note: The Hardy-Weinberg equilibrium analysis was performed with chi-square test.

Table 4: The distribution of the genotype in patients with normal or elevated serum AST levels

Genotype	Normal (n)	Elevated (n)	Unadjusted		Adjusted	
			OR (95% CI)	*P*	OR (95% CI)	*P*
GSTP1 rs1695						
AA	88	40	Reference		Reference	
AG+GG	56	10	0.39 (0.18-0.85)	**0.015**	0.37 (0.16-0.84)	**0.017**
PON1 rs662						
CC	59	27	Reference		Reference	
CT	61	19	0.68 (0.34-1.35)	0.271	0.74 (0.36-1.53)	0.422
TT	24	4	0.36 (0.12-1.15)	0.077	0.36 (0.11-1.16)	0.087
CT+TT	85	23	0.59 (0.31-1.13)	0.110	0.62 (0.32-1.23)	0.172
CAT rs769217						
CC	43	25	Reference		Reference	
CT	70	13	0.32 (0.15-0.69)	**0.003**	0.30 (0.13-0.68)	**0.004**
TT	31	12	0.67 (0.29-1.53)	0.335	0.66 (0.28-1.59)	0.359
CT+TT	101	25	0.43 (0.22-0.82)	**0.010**	0.41 (0.20-0.82)	**0.012**

Note: Each gene locus and the risk of abnormal liver function during VPA treatment was analyzed by unconditional logistic analysis method, balancing the confounding factors obtain odds ratio (OR) and 95% confidence interval (95% CI). P<0.05 has statistically significant.

The results showed that there was a statistical difference in body weight between the AST abnormal group and the AST normal group (P=0.008), and other clinical data such as gender, age, VPA dose, daily body weight dose, and VPA concentration were not statistically different between the AST normal group and the abnormal group.

Table 3 list the distribution and allelic frequency of each

SNPs genotype of the included patients with epilepsy, and the chi-square test showed that SNPs were in line with Hardy-Weinberg equilibrium.

Correlation between genetic polymorphisms and VPA-induced abnormal adverse reactions of AST

The distribution of the different genotypes of each SNP in the AST normal group and the abnormal group was analyzed, and the dominant genetic model was used to analyze the sites with less than 10 mutations homozygous. As shown in table 4, the patient with *GSTP1* rs1695 AG and GG genotype had a lower risk of AST abnormalities (Adjusted OR=0.37, 95% CI: 0.16-0.84, P=0.017). Carriers of CT genotype or CT+TT genotype of *CAT* rs769217 have a reduced risk of AST abnormalities (Adjusted OR=0.30, 95% CI: 0.13-0.68, P=0.004 and Adjusted OR=0.41, 95% CI: 0.20-0.82, P =0.012). There was no difference in the distribution of other SNP genotypes between the AST normal group and the abnormal group.

DISCUSSION

Based on the theory of "toxic metabolites" and "oxidative stress" of VPA-mediated hepatotoxicity, this study explored the correlation between the genetic polymorphisms in the key pathway of valproate oxidative stress and valproate-induced AST abnormalities in children. In children with epilepsy and treated with VPA monotherapy, *GSTP1* rs1695 and *CAT* rs769217 polymorphisms were associated with VPA-mediated AST abnormalities

A π isoenzyme in GSTs encoded by *GSTP1* is an important phase II metabolic enzyme *in vivo*. Its main functions are detoxification and protect DNA from the damage of free radical. The difference in catalytic efficiency between allelic variants at *GSTP1* rs1695 depends on the characters of the poison and the substrate selectivity of different enzyme forms. The enzyme activity encoded by the GG genotype of *GSTP1* rs1695 has higher catalytic activity for polycyclic aromatic hydrocarbon diol epoxides than the AA genotype (Sundberg *et al*., 1998). However, the enzyme activity encoded by AA type is higher than that of GG genotype when using 1-chloro-2,4-dinitrobenzene as the substrate (Hu *et al*., 1997). Reszka *et al*. found that the expression of *GSTP1* mRNA in the peripheral blood of subjects with *GSTP1* rs1695AA genotype was lower than that of subjects carrying the G allele, and the antioxidant activity of *GSTP1* in patients with AA genotype was significantly reduced(Reszka *et al*., 2011). *GSTP1* GG genotype plays a role in liver cancer susceptibility(Li *et al*., 2013b), and an Egyptian study found that carrying *GSTP1* G allele is associated with increased risk of liver cancer(Abo-Hashem *et al*., 2016) . A study found that the rs1695 A allele is associated with the susceptibility of anti-tuberculosis drugs to liver injury (Wu *et al*., 2016). He *et al*. found that the hypermethylation of the *GSTP1* promoter region is not only related to the liver injury susceptibility of anti-tuberculosis drugs, but also causes the decrease of *GSTP1* expression. *GSTP1* methylation is significantly associated with an increased risk of liver cancer (He *et al*., 2015). The organochlorine pesticides (OCPS) exposure of *GSTP1* GG genotype patients has a greater impact on the risk of liver cancer than genotype AA(Tian *et al*., 2020). In addition, children with cystic fibrosis carriers of the *GSTP1* rs1695 AA genotype are 8-folds more likely to develop liver disease than carriers of the G allele (Henrion-Caude *et al*., 2002). A rearch suggested that *GSTP1* genotype may be related to lung function in the first year after hematopoietic stem cell transplantation (SCT), patients with glutathione S-transferase P1 (*GSTP1* SNP rs1695) 1 or 2 minor alleles have lower lung function than those of homozygous ancestor alleles (Stark *et al*., 2017). In liver cancer patients, the survival time of GG genotype is longer than that of AA genotype (Li *et al*., 2012, Abo-Hashem *et al*., 2016). *GSTP1* rs1695 gene polymorphism also has an impact on obesity in young adults. Compared with AA genotype, G allele carriers are 2.4 times more likely to suffer from obesity than AA genotype (Chielle *et al*., 2017). Carrying *GSTP1* rs1695 polymorphism is related to the efficacy and adverse reactions of chemotherapy drugs in gastric cancer, colorectal cancer, non-small cell lung cancer, ovarian cancer, esophageal cancer and breast cancer(Sun *et al*., 2019, Reszka *et al*., 2011) . In this study, we found that children with epilepsy with *GSTP1* rs1695 AA genotype are more likely to have AST abnormalities during VPA monotherapy. VPA-induced liver toxicity is mainly based on the oxidative stress theory. The GST antioxidant activity of patients with AA genotype is lower, which reasonably explains the results of this study.

Some early studies have shown that individuals with CC alleles have higher levels of paraoxonase 1 in serum and better enzyme activity (Précourt *et al*., 2011). In 1996, Davies and other researchers found that *PON1* rs662 gene polymorphism affects the ability of paraoxonase 1 to catalyze certain substrates(Davies *et al*., 1996). Our study did not observe that the *PON1* rs662 gene polymorphism affects the risk of VPA-induced AST abnormalities.

Catalase (CAT) plays an important role in the biotransformation process of ROS, which can catalyze the conversion of H_2O_2 into water and prevent cell damage and apoptosis caused by ROS. *CAT* rs769217 C>T is a synonymous mutation in exon 9 of the *CAT* gene. Although the amino acid is not changed, the mutation at rs769217 can change the transcriptional activity of *CAT*. The mutation of 389C>T reduces the interaction between DNA and protein, and also inhibits the transcription of *CAT* gene (Yang *et al*., 2015). The T allele of *CAT* variants is associated with an increased risk of liver

fibrosis and hepatocellular carcinoma (Sousa *et al*., 2016). Liu *et al*. have found that the *CAT* rs769217 T allele can increase the risk of HBV-related chronic hepatitis, cirrhosis and liver cancer (Liu *et al*., 2015). In this study, the T allele at *CAT* rs769217 can reduce the risk of VPA-mediated AST abnormalities. This is contrary to the results of existing functional studies, so it needs more studies to verify it.

Our research still has the following limitations: The sample size of the study was small, not enough to find the correlation between SNP and VPA-induced AST abnormalities, leading to negative results. Due to the patient's voluntary willingness, the study's follow-up interval is uncertain, so it is hard to examine the effect of the course of medication on the elevation of VPA-mediated transaminase.

CONCLUSION

The results of the study on genetic polymorphisms and adverse reactions of VPA liver toxicity in children found that: Gene polymorphisms of key antioxidant enzymes have an impact on VPA-mediated AST abnormalities in children. We conducted epilepsy children with GSTP1 rs1695 G allele have a reduced risk of AST abnormalities, and CAT rs769217 CC genotype is a risk factor for AST elevation in children.

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