

# Effect of Genetic Polymorphisms of CYP2C19 on the Steady-state Serum Concentrations of Valproic Acid in Chinese Han Patients With Schizophrenia

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## Primary research

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# Abstract

Valproic acid is an anticonvulsant, which is also widely used for treating psychiatric disorders. Some clinical trials have demonstrated benefits of valproic acid augmentation therapy in schizophrenia. Interindividual variability in valproic acid dose and serum concentration may reflect functional consequences of genetic polymorphisms in genes encoding drug-metabolizing enzymes. The aim of this study was to determine the relationship between serum concentrations of valproic acid and single nucleotide polymorphisms of the *cytochrome P450 (CYP) 2C19* gene in patients with schizophrenia. All patients had been receiving fixed dose of valproic acid for at least 2 weeks. The daily doses were 0.5–1.5 g. No other drugs except olanzapine were coadministered. Serum concentrations of valproic acid were measured using the ultra-high performance liquid chromatography method with mass-spectrometric detection. The *CYP2C19* (*CYP2C19\*2 G681A* and *CYP2C19\*3 G636A*) genotypes were identified by real-time PCR analyses. The mean concentration/dose ratios of valproic acid was significantly higher in patients with 1 ( $P = 0.029$ ) or 2 ( $P = 0.007$ ) mutated alleles for *CYP2C19* than in those without mutated alleles. The mean concentration/dose ratios of valproic acid was significantly higher in patients with *CYP2C19 \*1/\*2* genotype ( $P = 0.029$ ) or *CYP2C19 \*2/\*3* genotype ( $P = 0.014$ ) than in those with *CYP2C12 \*1/\*1* genotype. The findings of this study suggest that *CYP2C19* genotypes play an important role in controlling steady-state serum concentrations of valproic acid in Chinese Han population.

## Introduction

Over the last 40 years, a variety of adjunctive treatments have been used to treat schizophrenia (G. W. Christison et al., 1991). These are often used in addition to antipsychotics, in an attempt to alleviate the symptoms of schizophrenia such as hallucinations and delusional beliefs, although they have been used instead of antipsychotics. Valproate /valproic acid has been used for people whose psychosis did not respond to traditional therapy. Valproic acid is traditionally used as an anticonvulsant drug and is also used for affective disorders, especially for the treatment of acute mania (A. Cipriani et al., 2013). Furthermore it is thought to have anti-aggressive effects and it may reduce impulsive behaviour, which might be useful for some people with schizophrenia (L. Citrome et al., 2000). There is large interindividual variability in its pharmacokinetics and pharmacodynamics (Y. Ghodke-Puranik et al., 2013, J. Methaneethorn, 2018). Therefore, its serum concentration needs to be monitored as a guide of dose adjustment during the course of therapy.

The enzyme *cytochrome P450 (CYP) 2C19* is involved in the metabolism of valproic acid. Several mutated alleles of the *CYP2C19* locus that cause decreased enzyme activity, that is, *CYP2C19\*2* (G681A, splicing defect) and *CYP2C19\*3* (G636A, W212X, premature stop codon) have been reported (K. Kurose et al., 2012, U. M. Zanger and M. Schwab, 2013). The steady-state serum concentration of valproic acid is significantly dependent on the mutated alleles. However, there are large overlaps in these steady-state serum concentrations among the different genotype groups and considerable interindividual variations in the values within each genotype. In previous published studies, body weight, age, gender and comedication had significant influence on VPA distribution volume (J. Ding et al., 2015, H. Nakashima et

al., 2015, N. Ogusu et al., 2014). Although various genetic polymorphisms were associated with the increase or decrease of VPA serum concentration (T. Budi et al., 2015, X. M. Chu et al., 2012, W. Feng et al., 2016, Y. Guo et al., 2012, C. C. Hung et al., 2011, D. Jiang et al., 2009, T. K. Kiang et al., 2006, S. Mei et al., 2017, L. Tan et al., 2010), only two study identified the quantitative relationship between genetic polymorphisms *CYP2C19* and VPA distribution volume in epileptic patients (D. Jiang et al, 2009, S. Mei et al., 2018).

Therefore, we aimed to investigate the effects of various factors including *CYP2C19* polymorphisms, age, gender, body mass index (BMI) and duration of schizophrenia on the steady-state serum concentrations of valproic acid in Chinese Han patients with schizophrenia, which might be useful for VPA dose adjustment in clinical practice.

## Materials And Methods

### Patients

The subjects were 139 Chinese Han patients with schizophrenia (52 males and 87 females) who all fulfilled the criteria for schizophrenia, according to the ICD-10. They were physically healthy without any history of substance abuse, neurological disorder, delirium, or dementia and without any clinically significant findings, including a clinical laboratory examination, electrocardiography, and electroencephalography. The mean  $\pm$  SD of age and BMI value were  $34.03 \pm 12.85$  years and  $23.80 \pm 3.77$ , respectively. 10 were smokers ( $\geq 10$  cigarettes/day), whereas the remainders were nonsmokers. None was a heavy drinker. This study was approved by the Ethics Committee of the Hangzhou 7<sup>th</sup> people's hospital, and all the patients had given written informed consent to participate in this study.

### Drug treatment

All the subjects had received valproic acid for at least 2 weeks. It has been shown that plasma concentrations of valproic acid reach steady state by 2 weeks after repeated oral administration. The daily dose was fixed and was given twice a day. The patients were receiving no drugs except olanzapine (2.5-20 mg/d). Female patients did not receive oral contraceptives. Patients' adherence was confirmed by the nursing staff or their families. Blood samples were taken at 8 AM.

### Analytical methods

Serum concentrations of valproic acid were measured using the ultra-high performance liquid chromatography method with mass-spectrometric detection by Zhejiang BIOZON Medical Co.,Ltd. The lowest limit of detection for valproic acid was 2.5  $\mu\text{g/ml}$  using 0.4 ml of serum, and the interassay coefficient of variation was 7.98% at a concentration of 50  $\mu\text{g/ml}$ . The steady-state serum concentrations was adjusted by the doses of valproic acid, and the concentration/dose (C/D) ratios were used in statistical analyses.

DNA was isolated from peripheral leucocytes using a nucleic acid extraction and purification kit (Kuangyuan, Suzhou, China). The \*2 and \*3 alleles of *CYP2C19* were identified by PCR analyses using Human *CYP2C19* genotyping kit (Kuangyuan, Suzhou, China).

## Statistical analyses

The serum C/D ratios of valproic acid of patients were compared among different genotypic groups or different metabolic type groups using one way ANOVA. Multiple regression analyses were used to detect the correlation between serum C/D ratios of valproic acid and several factors, including the polymorphisms of *CYP2C19*, age, gender, BMI values, smoke and duration of schizophrenia. A P value of less than 0.05 was regarded as statistically significant. SSPS 19.0 for Windows was used for these statistical analyses.

## Results

### Influence of *CYP2C19* genotype on serum C/D ratios of valproic acid

To explore the effects of *CYP2C19* genotype on interindividual variabilities in serum concentrations of valproic acid in Chinese Han schizophrenia, the *CYP2C19*\*2 (G681A, splicing defect) and *CYP2C19*\*3 (G636A, W212X, premature stop codon) were analyzed by real-time PCR. The characteristics of study population and the frequencies of *CYP2C19* genotype are shown in Table 1. There was no significant differences in the general characteristics among genotypes.

Table 1  
Characteristics of study population

	*1/*1	*2/*2	*3/*3	*1/*2	*1/*3	*2/*3	<i>p</i>
Male/female, n	22/34	5/9	0/0	21/37	2/4	2/3	0.770
Age, years	34.55 ± 12.39	30.71 ± 9.98	-	34.66 ± 14.04	28.50 ± 14.80	35.60 ± 12.18	0.683
BMI	23.58 ± 3.31	23.89 ± 2.63	-	23.69 ± 4.37	22.91 ± 2.80	23.88 ± 8.08	0.989
Duration of schizophrenia, month	123.29 ± 112.89	88.50 ± 80.71	-	126.55 ± 116.76	82.00 ± 55.47	182.40 ± 140.30	0.462

Furthermore, associations between *CYP2C19* SNPs and serum C/D ratios of valproic acid were observed. The number of patients with *CYP2C19* \*1/\*1, \*1/\*2, \*1/\*3, \*2/\*3, \*2/\*2 and \*3/\*3 genotype were 56, 58, 6, 5, 14 and 0, respectively (Table 2). Patients with *CYP2C19* \*1/\*2 (*P* = 0.029) or *CYP2C19* \*2/\*3 (*P* = 0.014) had significantly higher serum C/D ratios of valproic acid than those with *CYP2C19* \*1/\*1 (Table 2). And, the mean concentration/dose ratios of valproic acid was significantly higher in patients

with 1 (heterozygous extensive metabolizers,  $P = 0.029$ ) or 2 (poor metabolizers,  $P = 0.007$ ) mutated alleles for *CYP2C19* than in those without mutated alleles (Table 3 and Fig. 1).

Table 2  
Steady-state serum C/D Ratio of VPA between  
*CYP2C19* genotypes

Genotype	N	VPA C/D ratio ( $\mu\text{g/ml/g}$ )
*1/*1	56	94.61 $\pm$ 46.88
*2/*2	14	121.61 $\pm$ 51.90
*3/*3	0	-
*1/*2	58	114.61 $\pm$ 47.57 a
*1/*3	6	109.13 $\pm$ 34.11
*2/*3	5	150.96 $\pm$ 73.55 b
a $p < 0.05$ compared with *1/*1		
b $p < 0.05$ compared with *1/*1		

Table 3  
Steady-state serum C/D Ratio of VPA between *CYP2C19* metabolic  
types

metabolic types	N	VPA C/D ratio ( $\mu\text{g/ml/g}$ )
Homozygous EMs (*1/*1)	56	94.61 $\pm$ 46.88
heterozygous EMs (*1/*2 & *1/*3)	64	114.10 $\pm$ 46.29 a
PMs (*2/*2 & *2/*3 & *3/*3)	19	129.33 $\pm$ 57.65 b
a $p < 0.05$ compared with Homozygous EMs		
b $p < 0.01$ compared with Homozygous EMs		
EMs:extensive metabolizers; PMs:poor metabolizers		

## Multiple Regression Analysis

Multiple regression analysis including *CYP2C19* polymorphisms, age, gender, BMI values, smoke and duration of schizophrenia revealed that the polymorphisms of *CYP2C19* (standardized beta = 0.251,  $p < 0.01$ ) and the BMI values (standardized beta=-0.215,  $p < 0.01$ ) were correlated with C/D ratios of valproic acid (Table 4).

Table 4  
Standardized partial correlation coefficients (Beta) between serum C/D ratio of VPA and various factors (n = 139)

Variables	Beta	<i>p</i>
CYP2C19 genotype	0.251	0.003
Gender	-0.117	0.171
Age	0.185	0.064
BMI	-0.215	0.009
Smoke	-0.130	0.135
Duration of Schizophrenia	-0.143	0.151

## Discussion

Genetic polymorphism may be an important source of interindividual variability in the pharmacokinetics and pharmacodynamics of valproic acid. In this study, the mean serum C/D ratios of valproic acid increased in accordance with the number of the mutated alleles for *CYP2C19*. It is the data that strongly implied that the mean serum C/D ratios of valproic acid increases in a gene dose-dependent manner. The mean concentration/dose ratios of valproic acid were significantly higher in patients with 1 or 2 mutated alleles for *CYP2C19* than in those without mutated alleles. The result was assistant with previous report in epileptic (L. Citrome et al, 2000).

Otherwise, multiple regressin analyses showed that the serum C/D ratios of valproic acid were not only correlated with the number of mutated alleles for *CYP2C19* but also associated with the BMI values. One study showed that loss-of-function *CYP2C19* polymorphisms were associated with an increased BMI values (M. Noai et al., 2016). This may explained the association between BMI values and the serum C/D ratios of valproic acid in our study. And some studies reported the effect of age on the metabolism of valproic acid (U. A. Argikar and R. P. Remmel, 2009, S. J. Miyagi and A. C. Collier, 2011), showed that there was no statistically significant difference in the rate of VPA metabolism. Another study showed that serum valproic acid levels was significantly increased with younger age (L. Ben Mahmoud et al., 2017). These results are inconsistent. A larger population sample size is needed to further verify the results.

This study has several limitations. The dose of valproic acid was not unified. Data of clinical responses related with the observed differences in the steady-state serum concentrations of valproic acid were lacking. The possibility of committing type II errors cannot be ruled out because of the low power.

## Conclusions

The findings of this study suggest that *CYP2C19* genotypes play an important role in controlling the steady-state serum concentrations of valproic acid in Chinese Han subjects.

## Declarations

### Ethics approval and consent to participate

The study was approved by the Ethics Committee of the Hangzhou Seventh People's Hospital. The purpose and importance of the study were explained to each participant before they proceeded into actual activities. Informed consent was obtained from all participants.

### Consent for publication

Not applicable.

### Availability of data and material

All data generated or analyzed during this study are included in this published article.

### Competing interests

There are no conflicts of interest to report for any of the authors.

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### Authors' contributions

Wang S, Song M, Shi J were responsible for study design, Ju X, Liu J, Wang C, Shi J were responsible for recruiting the subjects, Wang S, Li J, Yan P, Ju X were responsible for collecting clinical data and performing the clinical rating. Wang S, Li J, Yan P were responsible for statistical analysis, and manuscript. All authors contributed to and have approved the final manuscript.

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## Figures

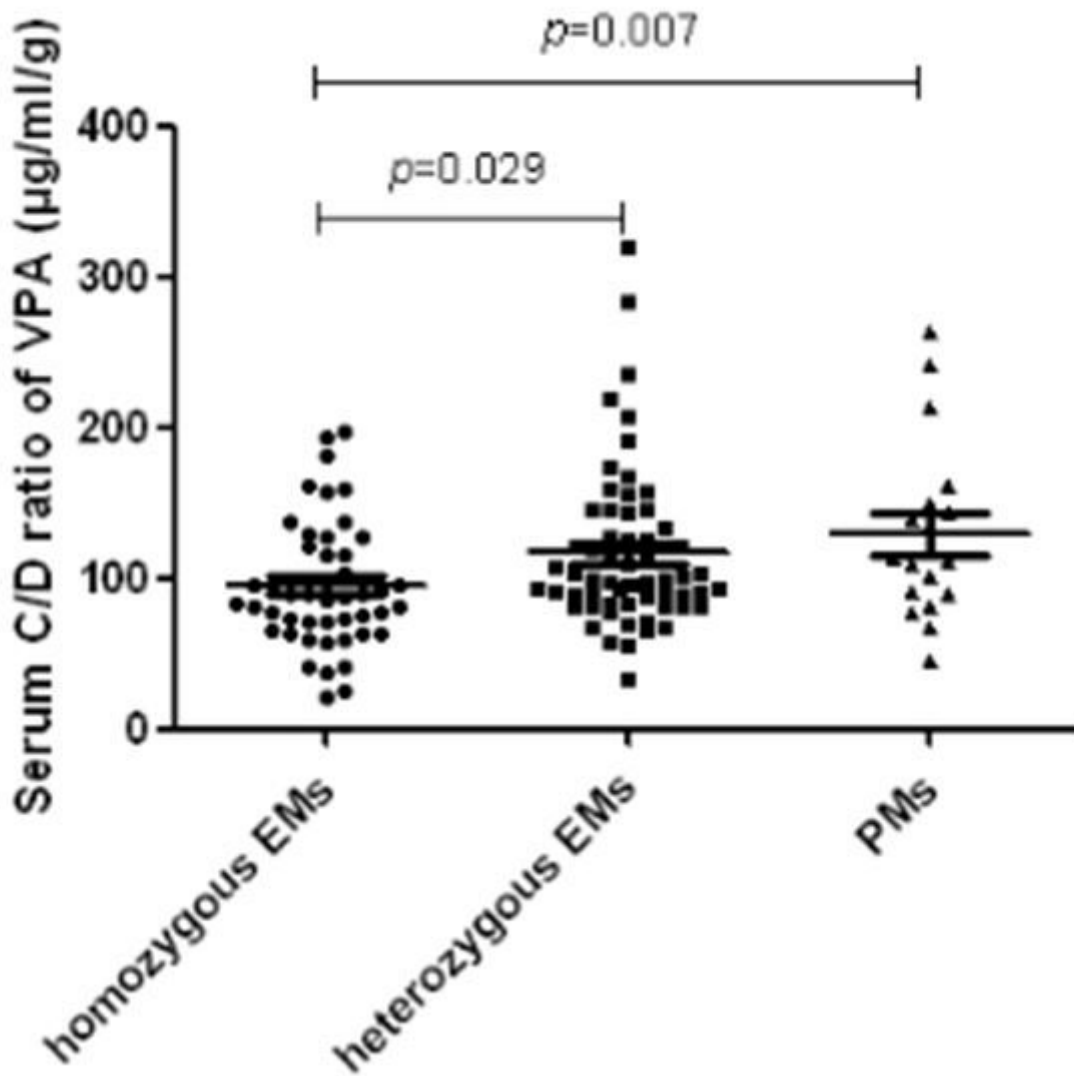


Figure 1

Differences in serum concentration/dose ratio of valproic acid in various metabolizer of CYP2C19 in Chinese schizophrenia patients. EMs: extensive metabolizers; PMs: poor metabolizers.