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ORIGINAL RESEARCH

Evaluation of *CYP2C19* Genetic Variant and Its Lack of Association with Valproic Acid Plasma Concentrations Among Zhuang and Han Schizophrenia Patients in Guangxi

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Purpose: To investigate the *CYP2C19* genotype distribution and allelic frequency among the Zhuang and Han schizophrenic populations in Guangxi, examine the correlation between *CYP2C19* genetic variants and standardized blood levels of Valproic Acid (VPA) in schizophrenic patients, and evaluate the effects of age, gender, and Body Mass Index (BMI) on standardized VPA blood concentrations.

Patients and Methods: Between February and December 2022, 192 Zhuang and Han schizophrenia patients treated with VPA were studied. Steady-state VPA concentrations were determined using homogeneous enzyme immunoassays, and *CYP2C19* *1, *2, and *3 loci via q-PCR. *CYP2C19* genotype distributions between Zhuang and Han groups in Nanning were compared using chi-square tests and contrasted with other ethnicities. Non-parametric tests analyzed VPA variations, identifying critical factors through multivariate stepwise regression.

Results: The study identified five *CYP2C19* genotypes at the *2 and *3 loci, with the *3/*3 genotype absent in both cohorts. The *CYP2C19* distribution in Guangxi Zhuang and Han mirrors, yet diverges significantly from Hui and Kazakh groups. Among 192 subjects, VPA blood levels remained consistent across metabolic types and ages 18–60 but varied significantly by gender. Multivariate analysis revealed gender and BMI as significant factors, overshadowing *CYP2C19* genotype and age.

Conclusion: In Guangxi, *CYP2C19* genetic variants in Zhuang and Han schizophrenia patients demonstrate statistically indistinguishable allelic and metabolic distributions. Gender and BMI can influence standardized VPA blood concentrations in schizophrenia patients. However, in our study cohort, the *CYP2C19* genotype and age are not the primary determinants of standardized VPA blood levels.

Keywords: *CYP2C19* gene, valproic acid, blood drug concentration, Zhuang Chinese, Han Chinese

Introduction

Pharmacogenomics delves into the intricate interplay between human genomic information and drug responses, with the ultimate goal of elucidating the variations in drug safety and efficacy among individuals.[1–3] As a pivotal facet of precision medicine, pharmacogenomics is centrally concerned with meticulous drug and dosage selection, targeted therapeutic interventions, and the anticipation of drug safety concerns, all while mitigating the emergence of deleterious side effects. This avenue of research empowers us to prognosticate patient responses to specific medications, thereby paving the way for bespoke treatment regimens.[4,5] The metabolism kinetics and therapeutic potency of drugs are frequently orchestrated by endogenous enzymes, under the orchestration of genes. In instances where distinct genetic

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variants manifest, their impact on drug effects and safety can be discernible. For instance, the *CYP2C19* gene encodes a member of the cytochrome P450 enzyme family, playing a pivotal role in drug metabolism.[6,7] This encompassing enzymatic responsibility extends to an array of pharmaceuticals, including certain antifungal agents, antidepressants, proton pump inhibitors, and antiplatelet aggregation medications.[8–11] The CYP2C19 enzyme, owing to its intricate genetic diversity, has emerged as a focal point in pharmacogenomic investigations, wherein these genetic variances may conceivably modulate enzyme functionality and drug metabolism.[12] Notably, the *CYP2C19* allele denoted as *CYP2C19**17, endowed with an augmenting influence on enzyme activity, might necessitate a commensurate escalation in drug dosages to attain therapeutic outcomes. Conversely, aberrant *CYP2C19* alleles, such as *CYP2C19**2 and *CYP2C19**3, could engender protracted metabolism, thus heightening the susceptibility to adverse effects, as expounded in the Clinical Pharmacogenetics Implementation Consortium (CPIC) dosing directives.[13]

VPA stands as an Anti-seizure medication (ASM), harnessed to address an array of seizure types, and extensively wielded in the management of generalized epilepsy. Beyond its epilepsy-related utility, VPA finds application in the realm of schizophrenia treatment. Operating as a mood modulator, it exerts governance over dopamine release and, in conjunction with other antipsychotic agents, orchestrates the containment of emotional volatility and impulsive tendencies. While VPA's therapeutic spectrum is broad, its margin of safety remains relatively slender. In accordance with consensus directives emanating from the Association for Neuropsychopharmacology and Pharmacopsychiatry (AGNP), the designated therapeutic reference interval for VPA blood concentration spans from 50 to 100 mg/L.[14] Dipping beneath this designated threshold might precipitate therapeutic inefficacy, whereas surpassing 120 mg/L could precipitate noteworthy adversities. Specifically, concentrations cresting 175 to 200 mg/L or free VPA levels eclipsing 14.67 mg/L may incite platelet diminution, culminating in purpura, hemorrhaging, and protracted clotting periods.[15,16] Noteworthy is the pronounced divergence in clinical dosages and blood VPA concentrations across individuals, with blood levels diverging by a factor of 2 to 2.5 even among recipients of identical doses.[17] These elements collectively curb the extent of VPA's clinical application. Albeit investigations underscore the role of the CYP2C19 enzyme in VPA metabolism,[18–20] and existing literature has probed into the ramifications of *CYP2C19* genetic variations on VPA blood concentrations within epilepsy patients,[21,22] a more comprehensive understanding of VPA metabolism necessitates consideration of additional CYP enzymes as indicated by the PharmGKB database (https://www.pharmgkb.org/pathway/PA165964265), which suggests that variants beyond *CYP2C19*, such as *CYP2C9, CYP2A6*, and *CYP2B6*, may also influence VPA plasma levels. The scope of studies delving into the impact of these genetic variations on VPA blood concentrations among schizophrenia patients remains circumscribed and inconclusive.

Prior investigations have illuminated disparities in the prevalence of *CYP2C19**2 and *CYP2C19**3 genetic variants across diverse geographical regions and ethnic backgrounds.[23] While genetic variations distributions of *CYP2C19* have been documented across distinct Chinese regions,[24–26] a comprehensive scrutiny of *CYP2C19* genetic variant distribution within the Zhuang and Han ethnic groups, specifically concerning individuals with schizophrenia in the Guangxi locale, remains an unexplored terrain. This study elucidates *CYP2C19* genetic variant distribution in Guangxi's Zhuang and Han cohorts, probing its correlation with VPA plasma levels in schizophrenia patients. Analyzing the influence of gender, age, and BMI on VPA concentration, our findings offer a foundation for individualized therapeutic strategies in clinical contexts.

Materials and Methods

Participants

The study encompassed a cohort of 192 inpatient individuals afflicted with schizophrenia, constituting 96 individuals of Zhuang ethnicity and an equivalent number of Han ethnicity, all of whom satisfied the established diagnostic criteria for schizophrenia. The eligibility criteria were defined as follows: (1) age spanning from 18 to 60 years; (2) confirmation of schizophrenia diagnosis in alignment with the guidelines stipulated by the International Classification of Diseases, Tenth Revision (ICD-10); (3) affiliation with either the Zhuang or Han ethnic groups; (4) unimpaired liver and kidney functionality; (5) no documented history of substance abuse; (6) alignment of demographic attributes encompassing gender and age between the two ethnic subgroups. Conversely, the exclusion criteria encompassed: (1) treatment-resistant schizophrenia; (2) expectant or nursing women; (3) Severe physical illnesses or organic brain disorders, neurological

disorders; (4) concurrent administration of carbamazepine, phenobarbital, or phenytoin. Notably, this investigation garnered approval from the Ethics Committee of the Fifth People's Hospital of Nanning City and was conducted in full compliance with the ethical standards of the Declaration of Helsinki. All participants provided informed consent prior to the collection of blood specimens, ensuring adherence to ethical guidelines (Approval Number: Ethics Review SL2022-02-03).

Drug Treatment

The patients underwent VPA therapy (Hunan Xiangzhong Pharmaceutical Co., Ltd) as part of a combination treatment, often required for schizophrenia, for a duration spanning at least 5 elimination half-lives, which approximates 100 hours. The VPA was administered at a prescribed dosage ranging from 0.2 to 1.0 grams per day, dispensed orally across two equitably spaced administrations. While VPA is used off-label for schizophrenia treatment and often accompanied by other antipsychotic medications, this study focused on VPA's role within the combination therapy. Any potential interaction effects were carefully monitored. Blood specimens were procured preceding the subsequent morning's fasted dose, ensuring a 12-hour interlude, to guarantee accurate assessment of steady-state trough concentrations.

Analytical Methods

The quantification of VPA serum levels was executed through employment of the Roche Cobas 702 fully automated biochemical analyzer in tandem with its corresponding reagents. The assay boasted a minimum detectable concentration of 2.8 milligrams per milliliter, characterized by a coefficient of variation (CV) less than 5%. To neutralize the potential impact of dosage and body weight on blood drug concentration, standardization of VPA concentration ensued as follows:

$$\text{Standardized VPA Concentration} = \text{Steady} - \text{state Drug Concentration}/\text{Daily Dose} \bullet \text{Body Weight}[\text{grams}/(\text{kilograms day})].$$

Isolation of DNA from blood samples was expedited with celerity, courtesy of a nucleic acid extraction kit extended by Suzhou Kuangyuan Biotechnology Co., Ltd. The genotyping of *CYP2C19* alleles, encompassing *2 and *3 variants, was consummated utilizing a human *CYP2C19* genotyping assay kit, also made available by Suzhou Kuangyuan Biotechnology Co., Ltd.

Statistical Analyses

Data entry procedures were conducted employing Excel 2013, whereas the realm of statistical analysis was navigated through utilization of the SPSS 25.0 software suite. The examination of genetic equilibrium in the context of Hardy-Weinberg equilibrium was accomplished through the chi-square goodness-of-fit test. As for continuous data, the scrutiny for normal distribution entailed the application of the Shapiro–Wilk test. Data diverging from normal distribution were succinctly portrayed as median values interposed within the interquartile range. Comparative analyses across groups were orchestrated using the Mann–Whitney *U*-test or Kruskal–Wallis test, depending on the nature of the data. Count data found their expression as frequencies or rates, and the chi-square test was employed to scrutinize discrepancies in allelic and metabolic frequencies across distinct ethnic clusters. In relation to VPA blood concentrations, subsequent to logarithmic transformation, the method of multivariate stepwise regression analysis was harnessed to holistically elucidate the nexus between diverse factors and standardized VPA blood concentrations. All statistical evaluations followed the trajectory of two-tailed probability assessments, and a level of significance set at $P<0.05$ denoted statistical significance.

Results

Patient Characteristics

The demographic characteristics of the patients are shown in Table 1. A total of 192 steady-state concentrations was collected. The plasma trough concentrations of VPA of the participants was 52.3 (39.2, 69.3) μg/mL and the median was within the 50~100 μg/mL therapeutic concentration of VPA. The standardized blood concentration of VPA was 2.30 (1.57, 3.51) μg/mL. Table 1.

Hardy-Weinberg Equilibrium Test for CYP2C19 Genotypes

CYP2C19 *2 and *3 genetic variants were consistent with Hardy–Weinberg equilibrium ($p>0.05$). Table 2.

Table 1 Demographic Characteristics of the Patients

Variable	Enrolled Patients (n=192)
Age, years M (P25, P75)	30.5 (22.25, 37.75)
Male sex, n (%)	95 (49.5%)
Female sex, n (%)	97 (50.5%)
BMI (kg/m2) M (P25, P75)	22.72 (19.93, 26.27)
Maintenance dose of VPA (g/day) M (P25, P75)	0.25 (0.25, 0.50)
Standardized VPA Concentration (μg/mL) M (P25, P75)	2.30 (1.57, 3.51)
Patients with VPA concentration <50 μg/mL, n	90
Patients with VPA concentration >100 μg/mL, n	5
Patients with co-medication, n (%)	50 (26%)
Co-medication days M (P25, P75)	49 (41.75, 57.25)

Abbreviations: M, Mean value; P25, 25th percentile; P75, 75th percentile; BMI, Body Mass Index; VPA, Valproic Acid.

Table 2 CYP2C19 Genetic Variant and Allele Distribution (n=192)

Genetic Variants	Genotypes			Allele Frequency	
	G/G	G/A	A/A	G	A
*2	89 (46.35%)	85 (44.27%)	18 (9.38%)	263 (68.49%)	121 (31.51%)
*3	175 (91.15%)	17 (8.85%)	0 (0)	367 (95.57%)	17 (4.23%)

Genotype Distribution and Allele Frequency of CYP2C19 Genetic Variants in Zhuang and Han Ethnic Groups

The genotype frequencies are shown in Table 3. The analysis of *CYP2C19* genotypes within the 192 patients revealed significant variances among the genotypes (*1/*1, *1/*2, *1/*3, *2/*2, 2/3) within both the Zhuang and Han ethnic clusters. Notably, neither group exhibited the *CYP2C19* 3/*3 genotype. The allele frequencies of *CYP2C19* *1, *2, and *3 in the Zhuang group were 63.54%, 31.77%, and 4.69% respectively, while in the Han group, they stood at 64.58%,

Table 3 Genotype Distribution and Allele Frequency of CYP2C19 Genetic Variants in Zhuang and Han Ethnic Groups

	Ethnicity		χ^2	*P*
	Zhuang	Han		
Genotype				
*1/*1	40 (41.67%)	40 (41.67%)	0.630	0.960
*1/*2	38 (39.58%)	39 (40.63%)		
*1/*3	4 (4.17%)	5 (5.21%)		
*2/*2	9 (9.37%)	9 (9.37%)		
*2/*3	5 (5.21%)	3 (3.12%)		
Allele				
*1	122 (63.54%)	124 (64.58%)	0.105	0.949
*2	61 (31.77%)	60 (31.25%)		
*3	9 (4.69%)	8 (4.17%)		
Metabolic phenotype				
NM	40 (41.67%)	40 (41.67%)	0.200	0.905
IM	42 (43.75%)	44 (45.83%)		
PM	14 (14.58%)	12 (12.50%)		

Abbreviations: NM, Normal metabolizer; IM, Intermediate metabolizer; PM, Poor metabolizer.

31.25%, and 4.17% respectively. Additionally, the assessment discerned three distinct CYP2C19 metabolic phenotypes: within the Zhuang group, Normal metabolizer (NM) constituted 41.67%, Intermediate metabolizer (IM) amounted to 43.75%, and Poor metabolizer (PM) accounted for 14.58%; in the Han group, NM represented 41.67%, IM tallied 45.83%, and PM encompassed 12.50%. Intriguingly, the distribution of *CYP2C19* genotypes, alleles, and metabolic phenotypes between the two ethnic groups bore no statistically significant disparities (P>0.05). Table 3.

Gender, Age and BMI Characteristics Among Different CYP2C19 Genotypes

The gender, age, and BMI characteristics among the 192 patients with different *CYP2C19* genotypes are presented in Table 4. There were no statistically significant differences in gender, age, and BMI among the various genotypes (P>0.05).

Comparison of CYP2C19 Allele Distribution and Metabolic Phenotype Distribution Between Zhuang Population in Guangxi and Various Population Groups in Other Regions of the Country

Amid the diverse Chinese regions and ethnicities, the mosaic of *CYP2C19* allele and metabolic phenotype distribution in Guangxi Han schizophrenia patients unveiled intricate patterns. Comparative analyses underscored noteworthy distinctions in *CYP2C19* allele distribution between Guangxi Han and Hui, as well as Kazakh populations (P<0.05). Similarly, the distribution of CYP2C19 metabolic phenotypes in Guangxi Han patients diverged significantly in contrast to Mongol, Hui, and Kazakh populations (P<0.05). Nevertheless, juxtapositions with Guangxi Zhuang, Dongguan, Huzhou, Hebei Han, Uighur, and Mongol populations yielded outcomes lacking statistical significance (P>0.05). Table 5.

The Influence of CYP2C19 Metabolic Phenotype, Age, and Gender on Standardized VPA Blood Concentration

The standardized VPA blood concentrations for the NM, IM, and PM metabolic phenotypes exhibited a gradual increase without statistical significance. Furthermore, while differences in blood concentrations among different age groups were not statistically significant, females had significantly higher blood concentrations than males, with statistical significance. Table 6.

Table 4 Gender, Age, and BMI Characteristics Among Different CYP2C19 Genotypes (n=192)

	*1/*1	*1/*2	*1/*3	*2/*2	*2/*3	χ^2/Z	P
Gender (M/F)	43/37	36/41	5/4	7/11	4/4	1.762	0.779
Age (years)	31.00 (22.25, 38.00)	30.00 (23.00, 37.00)	30.50 (24.50, 49.75)	28.00 (19.75, 41.25)	32.50 (21.25, 38.25)	0.200	0.938
BMI (kg·m-2)	22.84 (20.11, 25.56)	22.37 (20.17, 26.20)	22.51 (18.20, 27.17)	23.27 (19.93, 27.82)	19.68 (18.28, 26.14)	0.133	0.970

Table 5 Comparison of CYP2C19 Allele Distribution and Metabolic Phenotype Distribution Between Zhuang Population in Guangxi and Various Population Groups in Other Regions of the Country

Populations	Total Number	Allele Frequencies (%)			P	Metabolic Phenotype			P
	N	*1	*2	*3		NM	IM	PM	
Guangxi Han	96	64.58	31.25	4.17		41.67	45.83	12.50	
Guangxi Zhuang	96	63.54	31.77	4.69	0.959	41.67	43.75	14.58	0.905
Dongguan Han[27]	800	61.69	33.25	5.06	0.669	38.50	46.38	15.12	0.732
Huzhou Han[28]	678	61.87	32.45	5.68	0.615	38.35	47.05	14.60	0.769
Hebei Han[29]	451	67.29	27.94	4.77	0.636	43.68	47.23	9.09	0.590
Uighur[30]	214	65.42	32.48	2.10	0.344	40.19	50.47	9.35	0.617
Mongol[30]	158	54.11	41.46	4.43	0.061	26.58	50.06	18.35	0.039
Hui[30]	164	45.43	49.39	5.18	<0.01	19.51	51.83	28.66	<0.01
Kazakh[31]	107	76.64	15.42	7.49	<0.01	60.75	31.78	7.48	0.024

Table 6 Comparison of Standardized VPA Blood Drug Concentrations in Patients with Different CYP2C19 Metabolic Phenotypes, Age, and Gender

	Total	Standardized VPA Concentration (M (P25, P75), μg/mL)	Z	P
Metabolic phenotype				
NM	80	2.22 (1.51, 3.39)	0.257	0.880
IM	86	2.34 (1.56, 3.79)		
PM	26	2.45 (1.56, 3.45)		
Age (years)				
18~40	155	2.29 (1.54, 3.54)	0.313	0.754
41~60	37	2.63 (1.63, 3.43)		
Gender				
Male	95	1.94 (1.25, 2.68)	5.052	<0.001
Female	97	2.95 (1.99, 4.20)		

Multiple Stepwise Regression Analysis

The utilization of stepwise regression analysis culminated in the formulation of the regression equation for standardized VPA blood concentrations, incorporating gender and BMI: Standardized VPA blood concentration = 5.835–1.088×gender - 0.113×BMI (male = 1, female = 0). Notably, male blood concentrations trailed those of females (P<0.001), and BMI wielded a discernible influence on blood concentrations (P<0.001). However, the *CYP2C19* genotypes and age exhibited no discernible impact on blood concentrations (P>0.05). Table 7.

Discussion

The intricate orchestration of drug metabolism pivots upon the catalytic activity of diverse drug-metabolizing enzymes, among which the *CYP2C19* gene assumes a pivotal role by encoding pivotal drug-metabolizing enzymes.[32] Given the extensive clinical utilization of drugs that undergo metabolism facilitated by the CYP2C19 enzyme, the scrutiny of *CYP2C19* genotypes across different geographical regions and ethnic strata has assumed paramount importance. This trajectory of investigation offers a compass for prognosticating patient metabolic phenotypes and furnishing guidance for clinical drug administration.[33,34] Within the expanse of the *CYP2C19* gene, while an array of SNPs (single nucleotide genetic variants) has surfaced, the preeminent variant sites within the Chinese context involve *CYP2C19**2 and *CYP2C19**3. Within the ambit of our study, we embarked on a comprehensive analysis of the genetic variants manifested at these two allelic loci of the *CYP2C19* gene among Zhuang and Han ethnic individuals afflicted with schizophrenia within Guangxi. The findings unveiled a degree of congruence in the polymorphic distribution of the *CYP2C19* gene across the spectrum of patients with schizophrenia belonging to the Zhuang and Han ethnic clusters.

The congruence in the distribution of *CYP2C19* gene alleles within Guangxi's Zhuang and Han patients grappling with schizophrenia can be ascribed to the ethnic diversity underpinning the region. The continuum of long-term migration, intermarriage, and cultural interchange across disparate ethnic cohorts could be hypothesized to have contributed to the attenuation of genetic diversity between the Zhuang and Han populations. Furthermore, the shared living environments and cultural practices might have acted as catalysts for the semblance in gene distribution between

Table 7 Coefficient

Model		B	SE	Beta	t	P
2	(Constant)	5.835	0.466		12.532	<0.01
	Gender (Male = 1, Female = 0)	−1.088	0.182	−0.370	−5.965	<0.01
	BMI	−0.113	0.019	−0.367	−5.910	<0.01

the two groups. Our research findings align with those of studies conducted on Han populations in various regions such as Dongguan, Huzhou, Hebei, Xinjiang Uighurs, Guizhou, Hubei, Yunnan, and northern areas.[27–30,35–38] Nonetheless, significant disparities were detected in comparison with Hui and Kazakh populations, and distinctive disparities in gene phenotypes surfaced when juxtaposed with the Mongol population, especially in the Normal metabolizer (NM) phenotype.[30,31] These insights signify that while the variations in *CYP2C19* allele frequencies and metabolic phenotype distributions between Guangxi's Zhuang and Han populations do not substantially deviate from those observed across different segments of the Han populace, they do manifest marked disparities when compared to specific ethnic minorities. The mosaic of genetic diversity, geographical segregation, and influences of natural selection within China potentially contribute to divergent genetic profiles among distinct ethnic groups, mirroring evolutionary adaptations tailored to unique survival contexts. The existence of variants in the *CYP2C19* gene might reflect a survival advantage; nevertheless, these disparities warrant comprehensive contemplation within the context of drug treatment protocols. For instance, the CHANCE study underscored that in patients afflicted with mild ischemic stroke or transient ischemic attack (TIA), the concomitant administration of aspirin and clopidogrel yielded a reduction in stroke recurrence solely in patients devoid of the CYP2C19 loss-of-function allele, as contrasted with the administration of aspirin in isolation.[39] Additional clinical applications of *CYP2C19* genetic testing encompass the tailored prescription of selective serotonin reuptake inhibitors for depression treatment, the dosage adjustment of proton pump inhibitors for gastroesophageal reflux disease, and the calibration of voriconazole dosage for prophylaxis against invasive fungal infection, among other applications.[40]

CYP2C19 enzyme plays a vital role in metabolizing numerous drugs, including VPA, an antiepileptic and mood disorder medication. Nonfunctional or null alleles, like *CYP2C19**2 and *CYP2C19**3, can alter enzyme activity, influencing VPA blood concentrations.[21] Our study investigated the relationship between these genetic variants and standardized VPA blood levels, also considering the effects of gender, age, and BMI in schizophrenia patients. Contrary to our hypothesis, these genetic variations did not show a significant impact on VPA concentrations. Instead, gender and BMI appeared to play a notable role. These findings suggest implications for personalized treatment in schizophrenia and open doors for further research in pharmacogenomics.

The landscape of prior research presents a tapestry of inconsistent conclusions concerning the clinical ramifications of *CYP2C19* genotypes on VPA blood concentrations. For instance, Guo et al undertook a retrospective study encompassing 60 hospitalized patients, employing a nonlinear mixed-effects model to construct a population pharmacokinetic framework for VPA in Chinese patients. The foundational and ultimate objective function values for this model stood at 851.813 and 817.622, respectively, signifying a significant imprint of *CYP2C19* genotypes on VPA clearance rate.[41] However, a study by Smith et al involving 252 patients arrived at a contrary finding, unearthing no discernible linkage between *CYP2C19* genotypes and VPA blood concentrations.[22] Despite the trajectory of our study unveiling an inclination towards escalating standardized VPA blood concentrations across the NM, IM, and PM metabolic phenotypes, both univariate and multivariate analyses failed to unveil a substantive influence of *CYP2C19* genotypes on standardized VPA blood concentrations among patients grappling with schizophrenia. This phenomenon could potentially be attributed to the relatively modest VPA doses administered to the participants in our study. For instance, the investigation conducted by Wu et al comprising 122 bipolar disorder patients ascertained that it was only when VPA doses exceeded 1000 mg/d that the VPA concentrations among patients of the CYP2C19 NM phenotype trailed those of the IM subgroup.[42] In the realm of in vitro examinations, an array of uridine diphosphate glucuronosyltransferase (UGT) isoforms, encompassing UGT1A3, 1A4, 1A6, 1A8, 1A9, 1A10, 2B7, and 2B15, has been reported to partake in the process of VPA glucuronidation. Glucuronidation, alongside mitochondrial β-oxidation, comprises the dominant pathways governing adult VPA metabolism, while CYP2C19 occupies a relatively minor role.[43,44] We propose that CYP2C19-mediated oxidation becomes the primary determinant of serum VPA levels only when VPA concentrations surpass major metabolic pathways. In our study, the average maintenance dose was 0.25 (0.25, 0.50) grams per day, with only 3 of 192 patients receiving high doses. This limited high dose uses likely had minimal effect on CYP2C19 enzyme activity.

The shift in drug-metabolizing enzyme phenotypes potentially elucidates the disjunction between genotype-predicted metabolic phenotypes and the genuine capacity for individual drug metabolism, a disparity often engendered by non-genetic elements.[45,46] Investigations revolving around the classification of CYP2C19 activity have underscored a mere

40% alignment between genetically forecasted CYP2C19 phenotypes and actualized phenotypes, thereby unmasking the presence of phenotypic transformation.[47] Drug interactions with metabolic enzymes emerge as a prevailing factor instigating such metamorphoses, frequently perturbing enzyme activity via mechanisms of competitive inhibition. A vivid illustration can be found in scenarios involving lansoprazole and voriconazole treatment. Rigorous prospective investigations have brought to light gene-centric CYP2C19 inhibition effects, with NM and IM phenotypes culminating in a reduction of activity by a factor of 2.2-fold and 1.9-fold, respectively.[48] Moreover, the landscape of chronic liver disease is another variable that bears the potential to exert an impact on CYP2C19 activity. A forward-looking study delving into HCV-positive patients unearthed intricate interactions between genotype and the sphere of chronic liver disease.[49] The intricate interplay between inflammation and drug metabolism ratios is also worth noting. For instance, a meticulous prospective study encompassing 36 patients subjected to fluconazole treatment embarked on unraveling the repercussions of inflammation on fluconazole metabolism ratios.[50] Notably, a study by Kiss et al unearthed that CYP2C19 activity in individuals grappling with inflammatory ailments tended to be subpar when juxtaposed against the projected metabolic phenotypes.[46] The underlying mechanisms and extent to which the CYP2C19 metabolic enzyme is influenced remain largely ambiguous. Studies posit that diseases might induce a transformative shift in the CYP2C19 enzyme's phenotype. Specifically, in pathological conditions, when there's a surge in pro-inflammatory cytokines, particularly interleukin-6 (IL-6), it may trigger a selective inflammatory response that both inhibits the synthesis of the CYP2C19 enzyme and reduces its abundance, subsequently diminishing its metabolic efficacy.[51,52] The second-generation antipsychotic, Clozapine, may potentially harm mitochondria and incite the body to evoke a sophisticated inflammatory response, catalyzing the proliferation of pro-inflammatory cytokines such as IL-6, IL-17, GM-CSF, and IL12-p70, subsequently modulating their concentrations.[53,54] Consequently, the inflammation associated with Clozapine might instigate a phenotypical transformation of the drug metabolic enzyme. In psychiatry, especially among schizophrenia patients, the co-prescription of various medications is common. This can include combinations of antipsychotics, antidepressants, and drugs for physical conditions. Our study deliberately excluded drugs like phenytoin, carbamazepine, and phenobarbital, known to increase VPA's intrinsic clearance and alter its plasma concentration. However, how adjunctive drugs might affect the CYP2C19 enzyme is still unclear. The *CYP2C19* genotype is not the only factor in its metabolic profile. Such influences could usher in a disparity between genotype and phenotype, prompting phenotypical metamorphoses.[46] This might further illuminate the reason the *CYP2C19* gene's genetic variant did not markedly impact the normative VPA blood levels in the studied schizophrenia patients.

Additionally, laboratory experiments highlight that beyond CYP2C19, enzymes such as CYP2A6, CYP2B6, and CYP2C9 are integral to VPA's metabolic pathway. Specifically, CYP2C9 orchestrates 75–80% of VPA's terminal desaturation, its 4-hydroxylation, and 5-hydroxylation processes. CYP2A6 shoulders around half of the VPA 3-hydroxylation activity, with CYP2B6 governing the residual metabolic actions.[55–57] The internal metabolism of VPA is notably complex. Apart from undergoing Phase I oxidation through the CYP450 enzyme system, VPA also experiences mitochondrial β-oxidation and Phase II glucuronidation facilitated by UGT. Crucially, this Phase II glucuronidation emerges as an essential metabolic route for VPA, largely steered by UGT1A6, UGT1A8, and UGT2B7, encompassing about half of the provided VPA dose.[58–60] Consequently, one cannot dismiss the influence of CYP2A6, CYP2B6, CYP2C9, UGT1A6, and UGT2B7 on the metabolism of VPA.

Regression analysis from our study indicates no significant effect of age on VPA blood concentrations. This aligns with the population pharmacokinetic model established by Liu M X, wherein age is not deemed a factor influencing VPA blood concentration.[61] Yet, studies by Wang et al and Smith et al found increasing VPA concentrations with age, particularly in the elderly, where intrinsic clearance decreases by 40%.[22,62] The FDA drug label also advises reduced doses for the elderly due to decreased VPA clearance. These discrepancies may be linked to age-related metabolic activity of CYP2C19. Our study's age range was 18–60 years, excluding both minors and those above 65. This exclusion might be associated with the altered CYP2C19 activity in minors and changes in VPA metabolism in the elderly, impacting liver and kidney drug clearance.[63,64] Thus, the age effect on drug concentrations needs more comprehensive research in broader age ranges and larger samples.

Our research explores the gender-VPA blood concentration relationship. Females have about 30–50% less UGT activity for VPA daily dosages than males 59, potentially due to gender specific UGT1A gene control, as suggested by

Kalthoff et al.[65] Though VPA glucuronidation mainly happens in the liver, this does not fully explain the gender differences. Notably, females show a VPA bio-effective dose reabsorption rate of 46.2%, double that of males at 21.8%,[66] hinting at bioavailability discrepancies. This could explain gender specific VPA metabolism variations. Such insights might impact gender tailored VPA dosing. Additionally, our findings confirm that as BMI rises, standardized VPA blood concentration drops, aligning with other research.[21,67–69] This may result from the influence of body weight on VPA distribution volume: increased weight possibly expands this volume, leading to decreased blood concentrations 65. While certain *CYP2C19* alleles may slow VPA metabolism in epilepsy patients, weight gain might speed it up. This could be why we observed no significant influence of the *CYP2C19* genotype on VPA concentrations.

Our study acknowledges certain limitations, such as the exclusive focus on the *CYP2C19* *2 and *3 alleles and the absence of broader genetic screening for other CYP variants that may impact VPA metabolism. Additionally, metabolic factors such as insulin levels and lipid profiles were not assessed, which could contribute to a more comprehensive understanding of VPA's pharmacokinetics. Future studies should expand the genetic scope and integrate a wider metabolic assessment to refine personalized treatment strategies for schizophrenia.

Limitations

Owing to the clinical nature of this investigation, it was challenging to effectively regulate confounding variables that might influence drug metabolism, thereby leading to unavoidable limitations in the study outcomes. The research exclusively focused on the *2 and *3 allelic variants of *CYP2C19* to determine the impact of genetic variant on the VPA plasma concentration. However, other genetic loci warrant further exploration. Among the 192 schizophrenia patients in this study, only 17 exhibited variants at the *3 loci, and no instances of the *CYP2C19*3/*3 genotype were identified. This underscores that the correlation between this locus's SNPs and VPA metabolism still requires further validation.

Conclusion

In Guangxi, *CYP2C19* genetic variants are evident among the Zhuang and Han schizophrenia patients. The allelic frequencies, genotypes, and metabolic phenotypes are similarly distributed between the two ethnicities without statistical significance.

Gender and BMI can affect the standardized VPA blood concentrations in schizophrenia patients. Yet, in our study cohort of schizophrenia patients, the *CYP2C19* genotype and age are not the major determinants of standardized VPA blood levels.

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Disclosure

The authors report no conflicts of interest in this work.

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