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The Association Between Clozapine Plasma Concentration, CYP2D6 (*10, *2) Polymorphisms and Risk of Adverse Reactions

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ABSTRACT

Background: The aim of this article was to study the relationships between the risk of adverse reactions, plasma concentration, and cytochrome P450 2D6 rs1065852 (*10) and rs16947 (*2) polymorphisms for clozapine.

Methods: The steady-state clozapine plasma concentration of 100 Chinese inpatients with schizophrenia was determined using 2-dimensional liquid chromatography. The polymorphisms of cytochrome P450 2D6 (*10 and *2) were determined using fluorescent in situ hybridization protocols.

Results: The decreased percentages of white blood cells and neutrophils and the elevated percentages of creatine kinase, alanine aminotransferase, and aspartate transferase in patients treated with clozapine for 6 months were linearly associated with clozapine plasma concentration. Compared with the corresponding groups, the clozapine plasma concentrations of individuals with the *10TT genotype and individuals with the *2CC genotype were the highest ($P < .05$). The decreased percentages of white blood cells and neutrophils and elevated percentages of creatine kinase, alanine aminotransferase, and aspartate transferase for patients with the *10TT genotype were significantly higher than those for patients with the *10CC and *10CT genotypes ($P < .05$). The decreased percentages of white blood cells and neutrophils and increased percentages of creatine kinase, alanine aminotransferase, and aspartate transferase for patients with the *2CC genotype were significantly higher than those of the other groups ($P < .05$). The therapeutic reference range of clozapine for Chinese patients with schizophrenia was defined as 102.5-483.1 ng/mL.

Conclusion: This study demonstrated that the determination of cytochrome P450 2D6 polymorphisms and therapeutic drug monitoring of clozapine might be beneficial for identifying patients with a higher risk of adverse reactions.

ARTICLE HISTORY

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INTRODUCTION

Clozapine is an antipsychotic drug that is clinically effective in patients with schizophrenia who are resistant to other treatments.[1] Unfortunately, the application of clozapine is limited due to a number of undesirable aspects of its use. On the one hand, there are adverse reactions that are more frequent with clozapine than with other antipsychotics, including leukocytopenia, cardiotoxicity, hepatic injury, and so on. On the other hand, considerable variability in plasma concentration has been observed at the same dose, which is attributed to substantial interindividual differences in the metabolism of clozapine.[2] It has been reported that the clozapine plasma concentration rather than dose is associated with most of its side effects. However, further relationships between clozapine plasma concentration and adverse reactions have not been clarified completely. For instance, Tang et al[3] evaluated factors that might affect clozapine plasma levels in Chinese individuals; nonetheless, adverse reactions induced by clozapine plasma concentration were not clarified. Therefore, it is vital to investigate the relationships between clozapine plasma concentration and adverse reactions.

Recently, pharmacogenetics therapy has become more prevalent, reserving the strongest recommendations for therapeutic intervention of individuals. Cytochrome P450 (CYP) 2D6 is one of the most crucial drug metabolic enzymes and has been extensively studied. With high genetic polymorphisms, CYP2D6 is supposed to play a vital role in influencing the metabolic pathway and pharmacokinetic parameters of many antipsychotics, including clozapine.[4] The polymorphisms [rs1065852 (*10) and rs16947 (*2)] of CYP2D6 are associated with the efficacy and adverse

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reactions of antipsychotics.[5,6] The reports of Arranz et al[7] showed that the CYP2D6 gene appeared to be involved in a metabolic pathway for clozapine. However, the relevance of CYP2D6 (*10, *2) polymorphisms in Chinese patients with regard to the metabolism of clozapine remains unknown. Wicinski and Węclewicz reported that the pathogenesis of clozapine-induced agranulocytosis/granulocytopenia (CIAG) was a genetic aberration that was mainly caused by interactions of clozapine with CYP3A4 and CYP2D6.[8] However, further study of the relationship between CIAG and genetic polymorphisms of CYP2D6 was not discussed. In addition, the association between genetic polymorphisms of CYP2D6 and the impact of clozapine on cardiac and liver parameters has not yet been evaluated. Thus, illustrating the relationships between the genetic polymorphisms of CYP2D6 (*10 and *2) and adverse reactions is necessary.

Therefore, the goal of this study was to clarify the associations between clozapine plasma concentration and the risk of adverse reactions, including blood, cardiac, and hepatic reactions, in Chinese patients with schizophrenia during 6 months of clozapine treatment. Taking into consideration the interaction of CYP2D6 (*10 and *2) polymorphisms with clozapine, we also aimed to evaluate the potential influence of those interactions on clozapine plasma concentration and the risk of adverse reactions.

MATERIALS AND METHODS

Subjects

Based on the power calculation (expected power = 0.80), 100 Chinese patients with schizophrenia were enrolled. The inclusion criteria of patients were as follows: (1) patients receiving clozapine therapy for 6 months and (2) patients could use other antipsychotics or nonpsychiatric drugs not considered to affect clozapine metabolism and the examined indicators. The exclusion criteria were as follows: (1) obvious organic brain disease; (2) serious physical illnesses, such as heart, liver, or kidney diseases; (3) other diseases that might affect the examined parameters; and (4) use of other medications that might interfere with the metabolism of clozapine, such as carbamazepine. Clozapine therapy was given according to the conventional clinical protocol: initiation at a low dosage of 12.5-25 mg/day and subsequent titration in increments of approximately 12.5-50 mg/day until the optimal therapeutic effect was achieved. All patients provided informed consent before the study, in accordance with the Declaration of Helsinki. Ethical committee approval was received from the Ethics Committee of Wuxi Mental Health Center (WXMHC), protocol number of the present study was IRB2020LLKY004, and the date was September 20, 2020.

After excluding infections, blood samples of patients were collected after a 12-hour fast. Since clozapine has a half-life of 12-26 hours, the steady-state plasma concentration was ensured by maintaining subjects at the therapeutic dosage for at least 7 days.[3] Routine blood examination, including white blood cell (WBC) count, neutrophil (NEUT) count, platelet (PLT) count, lymphocyte (LY) count, and biochemistry tests, including creatine kinase (CK), alanine aminotransferase (ALT), and aspartate transferase (AST), were checked monthly from the first initiation of clozapine treatment.

Cytochrome P450 2D6 Genotyping

The polymorphisms of CYP2D6 (*10 and *2) were determined using fluorescent in situ hybridization protocols.[9] Blood samples were employed for DNA extraction using a QIAamp DNA Blood Mini Kit (Boao Biotechnology Co., Ltd, Beijing, China) following the manufacturer's instructions. The concentration of DNA was determined with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, NC, USA). The thermocycling conditions of 15 μL multiplex polymerase chain reaction (PCR) mixes were 5 minutes at 94°C followed by 40 cycles of 94°C for 30 seconds, 58°C for 20 seconds, and 72°C for 30 seconds, followed by a final incubation at 72°C for 30 seconds. To remove unincorporated deoxynucleoside triphosphates and PCR primers, the amplified products were purified by Exol and Fast AP. The extended products were analyzed using the RT-Cycler TM436/TL988 genetic analyzer (Boao Biotechnology Co., Ltd) after denaturation at 95°C for 3 minutes.

Determination of Clozapine Plasma Concentration

The clozapine plasma concentration of patients was determined by 2-dimensional liquid chromatography. Clozapine in 400 μL plasma was extracted by the addition of 1000 μL acetonitrile. After preparation, the aqueous layer was injected into the autosampler. Analysis of clozapine was performed with a C_{18} column (4.6 × 25 mm, 5 μm), a medium column (4.6 × 10 mm, 5 μm), and a phenyl column (4.6 × 100 mm, 5 μm). The ultraviolet detection was set at 260 nm. Representative chromatograms acquired from blank human plasma, clozapine-containing plasma, and patient plasma after the administration of clozapine are shown in Figure 1.

Statistical Analysis

All analyses were conducted by Statistical Package for the Social Sciences 22.0 (IBM SPSS Corp.; Armonk, NY, USA). The data were assessed using descriptive statistics (e.g., mean, frequency, and percentage). Descriptive statistics are given with mean ± SD and frequencies with percentages and median (Q1-Q3). Kruskal-Wallis test for non-parametric comparisons was used for non-normal variables. The statistics for different groups of demographic data, including plasma concentration and the changed levels of WBC, NEUT, PLT, LY, CK, ALT, and AST in

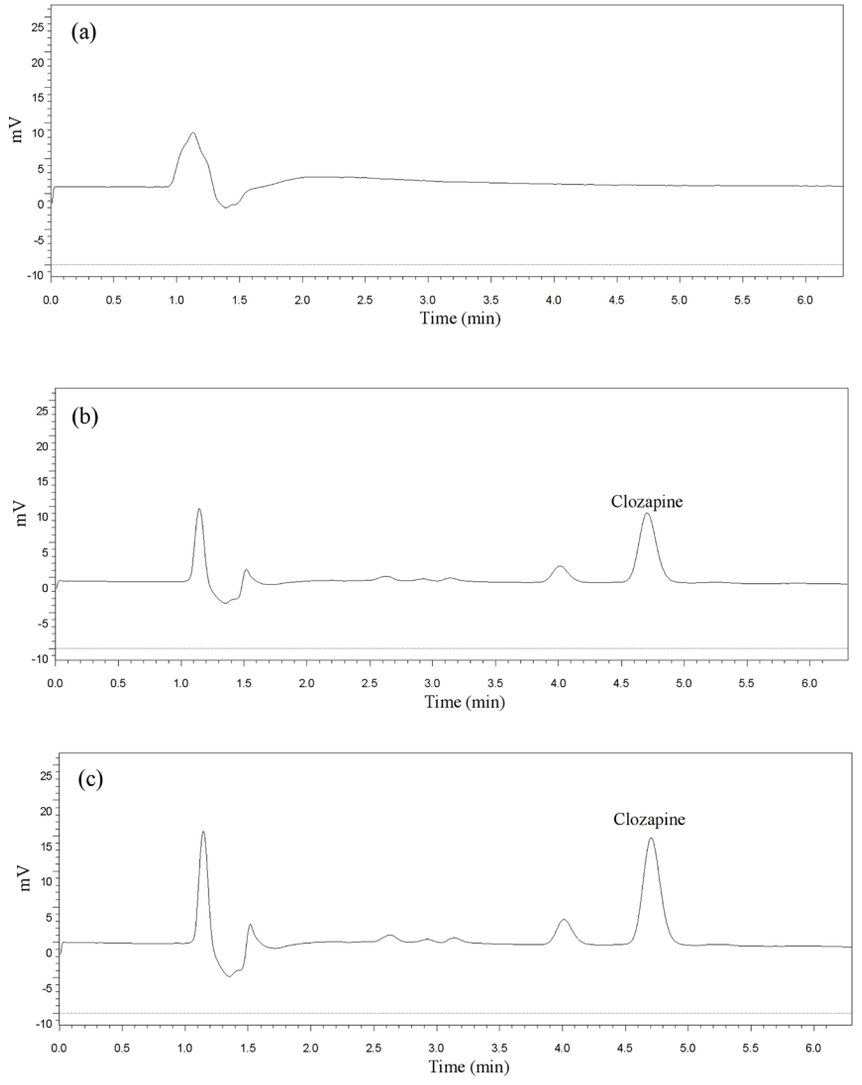

Figure 1. Representative chromatograms of clozapine. (A) Blank human plasma; (B) the clozapine-containing plasma; and (C) patient plasma treated with clozapine.

the *10 (CC, CT, and TT) and *2 (CC, CT, and TT) groups, were analyzed by 1-way ANOVA with Tukey post hoc test. The body mass index (BMI) of patients at baseline and after treatment for 6 months with clozapine was compared with the paired-samples t-test with Dunn–Bonferroni post hoc test. The Bayesian univariate and multiple linear regression analyses with enter method were conducted to determine the relationships between clozapine plasma concentration and the changed percentages of WBC, NEUT, PLT, LY, CK, ALT, and AST. $P < .05$ was considered significant.

RESULTS

The sociodemographic characteristics (e.g., gender, age, BMI, duration of illness, dose, and smoking status) of all the participating patients are listed in Table 1. The clinical data of patients in different groups are presented

Table 1. The Sociodemographic Characteristics of All the Patients

Characteristics		*P*
n	100	—
Gender		—
Male	79 (79.0%)	
Female	21 (21.0%)	
Age (years) (mean ± SD)	54.94 ± 12.19	—
BMI (kg/m^2)		
Baseline	22.67 ± 1.26	>.05
After treatment for 6 months	23.87 ± 1.34	
Duration of illness (years)	12.90 ± 7.11	
Average dose (mg)	184.75 ± 98.53	—
Smokers	15 (15.0%)	

BMI, body mass index.

Table 2. Distribution of CYP2D6 (*10, *2) Genotypes in Patients

SNP	HWE	Genotypes	n (%)	Gender (Male/ Female)	Smokers
CYP2D6*10	0.059	*10 CC	70 (70.00)	56/14	13
		*10 CT	23 (23.00)	20/3	2
		*10 TT	7 (7.00)	3/4	0
CYP2D6*2	0.98	*2 CC	18 (18.00)	12/6	1
		*2 CT	50 (50.00)	44/7	9
		*2 TT	32 (32.00)	24/8	5

CYP2D6, cytochrome P450 2D6; SNP: single nucleotide polymorphisms; HWE: hardy-weinberg equilibrium.

in Table 2. The significant differences for the changed percentages of WBC, NEUT, PLT, LY, CK, ALT, AST, and clozapine plasma concentrations among the *10 (CC, CT, and TT) and *2 (CC, CT, and TT) groups listed in Table 3 were symbolized. The results of the univariate linear regression analysis are shown in Table 4 ($P < .05$). $P < .05$ and $B > 0.4$ indicated that linear relationships exist between clozapine plasma concentration and the changed indices (e.g., WBC, NEUT, CK, ALT, and AST). The multiple linear regression analysis between plasma concentration and the changed percentages of WBC, NEUT, CK, ALT, and AST is shown in Figure 2 and Table 5 ($P < .05$). There were linear relationships based on the indices ($P < .05$, $B > 0.2$) of the multiple linear regression analysis. The genotype distribution of CYP2D6 (*10 and *2) conformed to Hardy-Weinberg equilibrium ($P = .059$ and $P = .980$, respectively). There was no significant difference in general clinical data, including gender, age, BMI, smoking status, and clozapine dosage, between the *10 (CC, CT, and TT) group and the *2 (CC, CT, and TT) group ($P > .05$).

The Changed Percentages of White Blood Cells, Neutrophils, Platelets, and Lymphocyte

Based on the univariate linear regression analysis, the decreased percentage of WBCs was linearly dependent on the clozapine plasma concentration (95% CI: 0.004-1.043, $R^2 = 0.185$, $P = .021$). Similarly, the percentage of NEUTs declined linearly with clozapine plasma concentration (95% CI: 0.035-1.770, $R^2 = 0.222$, $P = .007$). As shown in Table 3, the decreased percentages of WBCs and NEUTs in the *10 TT group were higher than those in the other 2 groups, which showed significant differences among the *10 (CC, CT, and TT) groups according to the post hoc test ($P = .001$, $P = .001$, respectively). The decreased percentages of WBCs and NEUTs in the *2 CC group were significantly higher than those of the other 2 groups among the *2 (CC, CT, and TT) groups ($P = .047$, $P = .021$, respectively) (Table 3).

Table 3. The Changed Percentage of WBC, NEUT, PLT, LY, CK, ALT, AST, and Clozapine Plasma Concentration in CYP2D6 (*10, *2) Different Groups

Index	CYP2D6*10			*P*	Post Hoc *P*	CYP2D6*2			*P*	Post Hoc *P*
	CC	CT	TT			CC	CT	TT		
Decreased percentage of WBC	10.91 (-4.07 to 24.37)	10.82 (-4.91 to 28.79)	38.28 (19.90-58.18)	.001*#▲	.001*#▲	18.79 (3.40-35.42)	8.42 (-7.34 to 24.49)	10.26 (-6.01 to 26.38)	.048*△ᵍ	.047*△ᵍ
Decreased percentage of NEUT	18.36 (1.41-36.97)	14.78 (0.78-29.57)	59.38 (39.87-78.96)	.001*#▲	.001*#▲	32.37 (7.01-57.98)	16.28 (-3.38 to 36.85)	21.45 (1.47-42.09)	.032*△ᵍ	.021*△ᵍ
Decreased percentage of PLT	3.55 ± 3.68	4.11 ± 3.23	3.36 ± 2.32	.981	.986	3.51 ± 2.11	3.96 ± 3.52	3.31 ± 4.07	.974	.981
Decreased percentage of LY	4.60 ± 3.50	5.58 ± 4.18	6.63 ± 2.81	.944	.950	4.85 ± 3.87	4.11 ± 4.45	5.51 ± 3.10	.942	.954
Increased percentage of CK	33.19 (-28.82 to 96.42)	44.31 (-9.67 to 100.43)	148.42 (46.31-256.59)	.001*#▲	.001*#▲	76.15 (-28.38 to 183.93)	26.32 (-70.26 to 123.94)	20.29 (-71.40 to 112.26)	.001*△ᵍ	.001*△ᵍ
Increased percentage of ALT	33.38 (-18.03 to 95.97)	40.04 (-16.05 to 97.64)	104.31 (46.31-162.59)	.001*#▲	.001*#▲	73.38 (-41.38 to 189.80)	44.35 (-27.25 o 117.57)	52.12 (-15.03 to 119.91)	.032*△ᵍ	.034*△ᵍ
Increased percentage of AST	12.41 (-21.03 to 46.64)	18.58 (-13.16 to 51.44)	151.47 (73.05-234.39)	.001*#▲	.001*#▲	70.42 (-31.02 to 172.09)	17.01 (-55.38 to 89.56)	21.32 (-49.53 to 92.92)	.043*△ᵍ	.046*△ᵍ
Clozapine plasma concentration	207.30 ± 137.09	214.30 ± 138.80	393.60 ± 140.75	.021#▲	.015#▲	332.49 ± 141.58	183.30 ± 131.03	216.08 ± 134.94	.005△ᵍ	.004△ᵍ

*$P < .05$ significant among CC, CT, and TT groups.
#$P < .05$ significant among *10CC and *10TT groups.
▲$P < .05$ significant among *10CT and *10TT groups.
△$P < .05$ significant among *2CC and *2CT groups.
ᵍ$P < .05$ significant among *2CC and *2TT groups.
ALT, alanine aminotransferase; AST, aspartate transferase; CK, creatine kinase; CYP2D6, cytochrome P450 2D6; LY, lymphocyte; NEUT, neutrophil; PLT, platelet; WBC, white blood cell.

Table 4. Statistical Results of the Univariate Linear Regression Analysis

Index	*B*	*P*	R^2	95% CI
WBC	0.501	.021*	0.185	0.004 to 1.043
NEUT	0.471	.007*	0.222	0.035 to 1.770
CK	0.524	.011*	0.275	0.192 to 1.377
ALT	0.485	.039*	0.236	0.138 to 1.295
AST	0.534	.001*	0.285	0.189 to 1.365
Smoke	0.013	.070	0.116	-0.136 to 1.023

*$P < .05$.
ALT, alanine aminotransferase; AST, aspartate transferase; CK, creatine kinase; NEUT, neutrophil; WBC, white blood cell.

There was no relationship between the changed percentage of PLTs or LYs and the plasma concentration of clozapine analyzed by univariate linear regression analysis ($P > .05$). In the post hoc analysis, no significant difference was found in the changed level of PLT or LY for patients in the *10 (CC, CT, and TT) groups ($P = .986$, $P = .950$, respectively) and *2 (CC, CT, and TT) groups ($P = .981$, $P = .954$, respectively).

The Changed Level of Creatine Kinase

The elevated percentage of CK was found to be linearly dependent on the clozapine plasma concentration (95% CI: 0.192-1.337, $R^2 = 0.275$, $P = .011$). In Table 3, the increased percentage of CK in the *10TT group was higher than those in the *10CC and *10CT groups, with a significant difference according to the post hoc test ($P = .001$). Compared with the *2 CT and *2 TT groups, the elevated percentage of CK in the *2CC group was significantly higher, and it was the highest among all groups ($P = .001$) (Table 3).

Table 5. Statistical Results of the Multiple Linear Regression Analysis

Index	*B*	*P*	Adjusted R^2	95% CI
WBC	0.229	.025*	0.427	-3.048 to 1.528
NEUT	0.305	.002*	0.305	0.998-4.118
CK	0.313	.005*	0.313	0.179-1.974
ALT	0.284	.047*	0.412	-0.062 to 1.886
AST	0.311	.035*	0.215	-0.264 to 1.693

*$P < .05$.
ALT, alanine aminotransferase; AST, aspartate transferase; CK, creatine kinase; NEUT, neutrophil; WBC, white blood cell.

The Changed Percentages of Alanine Aminotransferase and Aspartate Transferase

The increased percentages of ALT and AST were linearly dependent on the clozapine plasma concentration (95% CI for ALT: 0.138-1.295, $R^2 = 0.236$, $P = .039$; 95% CI for AST: 0.189-1.365, $R^2 = 0.285$, $P = .001$). As shown in Table 3, the increased percentages of ALT and AST in the *10 TT group were higher than that in the *10 CC or *10 CT groups according to the post hoc test ($P = .001$, .001, respectively). Compared with the *2 CT and *2 TT groups, the increased percentages of ALT and AST in the *2 CC group were the highest ($P = .034$, $P = .046$, respectively) (Table 3).

Clozapine Plasma Concentration and Smoking

The clozapine plasma concentration of females (360.9 ± 226.4 ng/mL) was significantly higher than that of males (185.0 ± 121.5 ng/mL) ($P = .001$). As presented in Figure 2, according to the quartiles method ($P_{25.0}$-$P_{75.0}$) as well as the multiple linear regression analysis (the clozapine plasma

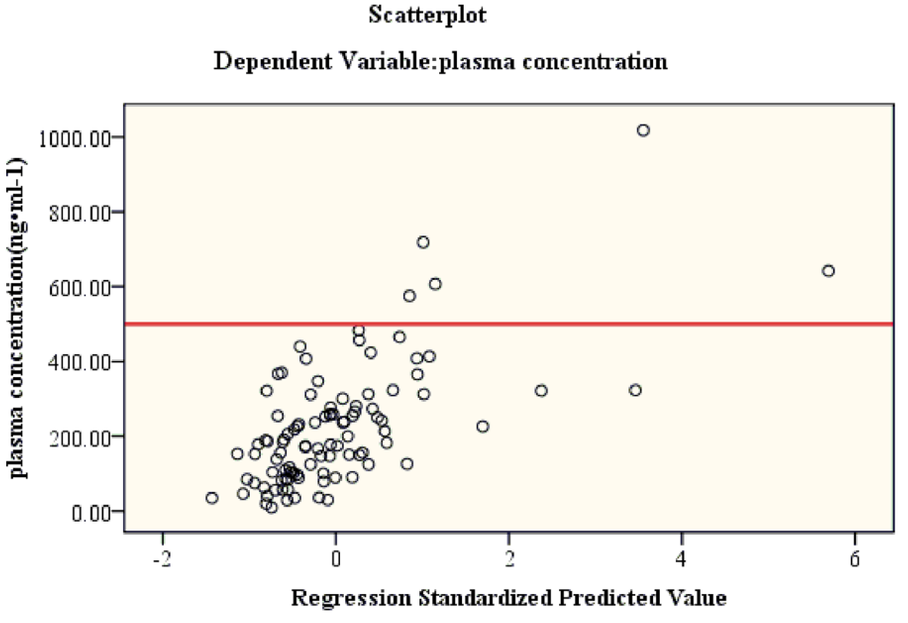

Figure 2. The multiple linear regression analysis between plasma concentration and the changed percentage of white blood cells, neutrophils, creatine kinase, alanine aminotransferase, and aspartate transferase ($P < .05$).

concentration was set as the dependent variable, while the changed percentages of WBC, NEUT, CK, ALT, and AST were set as independent variables) ($P < .05$), the therapeutic reference range in Chinese patients with schizophrenia was defined as 102.5-483.1 ng/mL, and, as a result, when the plasma concentration was above 483.1 ng/mL, the level of indices changed remarkably.

There was a negative relationship between smokers and the ratio of clozapine plasma concentration to dosage analyzed by univariate linear regression analysis (95% CI: -0.136--1.023, $R^2 = 0.116$, $P = .070$). However, in the *10 (CC, CT, and TT) and *2 (CC, CT, and TT) groups, there was no significant difference for clozapine plasma concentration between smokers and nonsmokers according to the post hoc test ($P > .05$).

Controlling for gender and smoking, the plasma concentration of the *10 TT group was the highest compared with the *10CT and *10TT groups according to the post hoc test ($P = .015$) (Table 3). Similarly, the plasma concentration of the *2 CC group was the highest compared with the *2CT and *2TT groups ($P = .004$) (Table 3).

DISCUSSION

The Influence on the Percentage of White Blood Cells and Neutrophils

This study highlighted that the decreased percentages of WBCs and NEUTs were weak linearly dependent on clozapine plasma concentration based on lower regression coefficients, indicating the importance of therapeutic drug monitoring (TDM) for clozapine. The decreased percentages of WBCs and NEUTs were more remarkable in the *10 TT and *2 CC groups, indicating that the WBC and NEUT counts of individuals with the *10 TT or *2 CC CYP2D6 genotype should be monitored.

Thus, in clinical practice, the TDM of clozapine and CYP2D6 (*10, *2) genotypes should be given close attention, and regular monitoring, including blood counts, should be performed to allow for early identification of falling blood counts as a predictor of leukocytopenia and neutropenia. In particular, when the clozapine plasma concentration in Chinese patients surpasses 483.1 ng/mL, the risk of WBC and NEUT counts falling is significantly increased. If WBC $< 4 \times 10^9$/L or NEUT $< 1.5 \times 10^9$/L, clozapine rechallenge should be evaluated by prescriber and hematologist consultation to determine whether the benefits outweigh the risks.[10]

The Influence on Creatine Kinase

Myocarditis or pericarditis, which can be fatal if not recognized and managed in a timely manner, may be relevant to clozapine.[11] The level of CK in plasma is the signal for the degree of heart injury when the heart is exposed. It has been reported that the risk factor for cardiac complications of clozapine might be rapid dose titration.[12] However, in this study, the regression coefficients indicated the existence of a weak linear relationship between CK and clozapine plasma concentration. The increased percentage of CK was in direct proportion to the clozapine plasma concentration, namely, if the clozapine plasma concentration of patients increased, the elevated percentage of CK rose linearly. Once the plasma concentration is limited to 483.1 ng/mL, the increase in the percentage of CK can be kept to a minimum, and, as a result, the occurrence rate of some diseases, such as myocarditis or heart failure, would be decreased. In this study, individuals with *10 TT and *2 CC genotypes were more likely to develop an increased percentage of CK; thus, they require extra attention when being treated with clozapine. Our findings illustrated that hereditary factors had a vital function on the risk of adverse reactions induced by clozapine.

Therefore, if clozapine treatment is necessary, CYP2D6 (*10 and *2) genetic polymorphisms of patients should be monitored before administering the medication. If a patient has the *10 TT or *2 CC genotype and a clozapine plasma concentration above 483.1 ng/mL, the risk of occurrence of increased CK percentage may be higher; thus, patients in this situation require close observation. When cardiac complications, such as myocarditis or pericarditis, are suspected or diagnosed, clozapine cessation is usually mandatory.[13] In addition, relieving medications, such as β blockers or angiotensin-converting enzyme inhibitors, could be utilized to remit the degree of injury.[14]

The Influence on Alanine Aminotransferase and Aspartate Transferase

There is an asymptomatic rise in serum transaminase levels for 30%-50% of patients treated with clozapine.[15] However, the exact incidence of hepatotoxicity induced by clozapine remains uncertain, and only a few studies have proposed that drug overdose or idiosyncratic drug reactions could be the leading cause of hepatotoxicity in North America.[16] Some studies have insisted that patients who experience liver injury with any drugs are more vulnerable to clozapine-induced liver injury.[17] This study had the surprising finding that clozapine plasma concentration had a weak linear influence on the hepatic indices based on regression coefficients, which was inconsistent with previous studies. Once the clozapine plasma concentration was above 483.1 ng/mL, patients undergoing clozapine therapy were more vulnerable to transaminitis. Compared with previous studies, these findings are more available and serviceable to clinical practice, indicating the importance of conducting TDM for patients treated with clozapine.

Our results clearly demonstrated the association of CYP2D6 (*10 and *2) polymorphisms with the risk of transaminitis and the possible involvement of the *10 TT and *2 CC genotypes in the risk of transaminitis. A clozapine plasma

concentration >483.1 ng/mL and the presence of the CYP2D6 genotype *10 TT or *2 CC may be the earliest indicators of the risk of elevated liver enzymes.

Clozapine Plasma Concentration and Smoking

There is evidence that clozapine plasma concentrations between 350 ng/mL and 400 ng/mL are not only associated with clinical response but also with markedly increased side effect burden.[10,18]. In our dataset, the clozapine therapeutic reference range of Chinese patients with schizophrenia was recommended to be limited to 102.5-483.1 ng/mL, and, as a result, the incidence rate of decreased percentages of WBC and NEUT counts and increased percentages of CK, ALT, and AST could be kept to a minimum. However, Hiemke et al[19] reported that the maximum plasma concentration of clozapine was 600 ng/mL, which was inconsistent with the present study. The possible reasons for this discrepancy are as follows: first, different ethnicities were included in those reports and our study. The previous study focused on the population of Europe and the USA, whereas Chinese patients were selected as the subjects in our study. Second, in this study, adverse reactions, including decreased percentages of WBCs and NEUTs and increased percentages of CK, ALT, and AST, were considered complete. However, only the risk of seizures was selected as the evaluated index in previous reports. Therefore, our findings were more credible than earlier reports for Chinese patients undergoing treatment with clozapine. However, further studies should be carried out to verify our results.

Mayerova et al[20] reported that smoking showed a negative correlation with the clozapine plasma concentration. However, in our study, the relationship between smoking and the ratio of clozapine plasma concentration to dosage was investigated. The clozapine plasma concentration was adjusted by daily dosage, which was more credible. Taking into consideration the influence of smoking on strong interindividual variability, the TDM of clozapine could be a useful approach for smokers.[20]

There was no significant relationship between CYP2D6 (*10 and *2) polymorphisms and the clozapine plasma concentration of smokers, demonstrating that CYP2D6 (*10 and *2) polymorphisms had no influence on the clozapine plasma concentration of smokers. The reasons for this phenomenon might be as follows: polycyclic aromatic hydrocarbons in cigarettes are induced by CYP1A2[21] rather than by CYP2D6.

The present study illustrated that individuals with the *10 TT or *2 CC genotype are more vulnerable to higher clozapine plasma concentrations. However, Katalin et al[22] reported that CYP2D6 seemed to have no effect on clozapine concentration in 92 Hungarian patients, which was opposite to our findings. The different ethnicities are the probable reason for this discrepancy.

The limitation of this study was its small sample size; large-sample, multicenter, and long-term clinical observations are necessary to confirm the conclusions of this study in the future. Although a lack of clinical responses was another limitation, our findings were inconsistent with those from previous studies.

This is the first report on the associations between clozapine plasma concentration, CYP2D6 polymorphisms, and changes in the levels of WBC, NEUT, CK, ALT, and AST. There were linear relationships between clozapine plasma concentration and adverse reactions, including decreased percentages of WBCs and NEUTs and increased percentages of CK, ALT, and AST. Individuals with the *10 TT or *2 CC genotype were more vulnerable to increased clozapine plasma concentration, increased levels of CK, ALT, and AST, and decreased levels of WBCs and NEUTs. Therefore, TDM for clozapine along with genotype detection of CYP2D6 (*10 and *2) is strongly recommended to avoid the risk of toxication. If the clozapine plasma concentration approaches the alert value (483.1 ng/mL) or the individuals has the *10 TT or *2 CC genotype, close attention should be paid to routine blood and biochemical tests. These findings could facilitate the improvement of individual treatment, leading to effective avoidance of adverse reactions to clozapine.

Ethics Committee Approval: This study was approved by Ethics Committee of Wuxi Mental Health Center (Approval No:IRB2020LLKY004, Date: September 20, 2020).

Informed Consent: Verbal informed consent was obtained from the patients who agreed to take part in the study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – K.Q.; Design – K.Q.; Supervision – Z.Z., Y.S.; Resources – L.T.; Materials – X.S.; Data Collection and/or Processing – Q.Z; Analysis and/or Interpretation – K.Q.; Literature Search – H.Z.; Writing – K.Q; Critical Review – Z. Z.

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