

Effects of *CYP3A4* polymorphisms on the plasma concentration of voriconazole

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Abstract Voriconazole is frequently utilized for the prevention and treatment of invasive fungal infections (IFIs), and is extensively metabolized by the cytochrome P450 (CYP) system. The impact of activity of the genes encoding *CYP3A4*, *CYP3A5*, and *CYP2C9* on the pharmacokinetics of voriconazole cannot be ignored because, second to *CYP2C19*, they are the most important enzymes involved in voriconazole metabolism. The influence of genetic polymorphisms in *CYP3A4*, *CYP3A5*, and *CYP2C9* on the plasma concentrations of voriconazole was evaluated in the present study. The study cohort comprised 158 patients with IFIs in whom 22 single-nucleotide polymorphisms (SNPs) in *CYP3A4*, *CYP3A5*, and *CYP2C9* were genotyped using the Sequenom MassARRAY RS1000 system, and voriconazole plasma concentrations were measured by high-performance liquid chromatography (HPLC). 40, 91, and 27 patients presented with low (<1 mg/L), normal (1–4 mg/L), and high (>4 mg/L) plasma voriconazole concentrations, respectively. Correlation analysis between polymorphisms and the plasma voriconazole concentration revealed an association between the presence of the rs4646437 T allele and a higher plasma voriconazole concentration [$p=0.033$, odds ratio (OR)=2.832, 95 % confidence interval (CI)=1.086–7.384]. This study has identified a new SNP related to the metabolism of voriconazole, potentially providing novel insight into the influence of *CYP3A4* on the pharmacokinetics of this antifungal agent.

Introduction

Voriconazole is a broad-spectrum triazole antifungal agent that is available as both an oral and an intravenous formulation, with potent in vitro and in vivo activity against *Aspergillus* and fluconazole-resistant *Candida* and *Fusarium* species [1, 2], and it is used mainly to treat and prevent invasive fungal infections (IFIs) in clinical practice. Data obtained from a meta-analysis study suggested a target plasma concentration range of 1–4 mg/L [3]. Plasma voriconazole concentrations demonstrate a large variability, with concentrations ranging from 0.2 to 12 mg/L, having been observed in patients with IFIs who were treated according to the recommended dosage regimen [4–7]. Modulators of this variability include both comedication and genetic factors [8–13]. Low voriconazole trough concentrations have been associated with poor treatment outcome, while high concentrations are suggestive of an underlying predisposition toward toxicity [14, 15]; both of these situations should, therefore, be avoided.

Voriconazole is extensively metabolized by the cytochrome P450 (CYP) system. N-oxidation of voriconazole into its major circulating metabolite is performed mainly by the cytochrome *CYP2C19* [8, 16]. It has been demonstrated that the variability of plasma voriconazole concentrations is related to the presence of *CYP2C19* polymorphisms, and that the most common defective alleles are *CYP2C19*2*, *CYP2C19*3*, *CYP2C19*4*, and *CYP2C19*17*. The frequency of occurrence of the *CYP2C19*4* allele is very low in Asians [8]. *CYP2C19* genotype status affects the metabolic rate as follows: *CYP2C19*1*17*: ultrarapid metabolizer; *CYP2C19*1*1*: extensive metabolizer; *CYP2C19*1*2* and *CYP2C19*1*3*: intermediate metabolizer; and *CYP2C19*2*2*, *CYP2C19*2*3*, and *CYP2C19*3*3*: poor metabolizer [17].

A final conclusion has not yet been reached as to the effects of polymorphisms in *CYP2C9* and *CYP3A4* on the plasma voriconazole concentration, although the association between

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variants of CYP3A4 and CYP2C9 and voriconazole pharmacokinetics have been studied, especially for some missense mutations [13, 18, 19]. Since CYP2C9 and CYP3A4 are also involved in the metabolism of voriconazole, the impacts of CYP3A4 and CYP2C9 activity cannot be ignored. In addition, another major member of the CYP3A family, CYP3A5, like CYP3A4, also plays an important role in drug metabolism [20], and some studies have demonstrated an association between this CYP and the physiological disposition of voriconazole [13, 19, 21]. It is, therefore, rational to hypothesize that single-nucleotide polymorphisms (SNPs) in CYP2C9, CYP3A4, and CYP3A5 could influence their activities and, further, affect the plasma voriconazole concentration.

In the present study, SNPs were selected according to the Chinese Han population data from HapMap and a literature search, and their association with the plasma voriconazole concentration was evaluated, taking into account other factors that influence voriconazole pharmacokinetics, with a view to providing some insight into voriconazole metabolism and pharmacokinetics.

Materials and methods

Study population

Chinese patients with a proven or probable IFI and who had received intravenous or oral voriconazole at loading or maintenance doses based on the package insert between January 2008 and August 2012 at the First Affiliated Hospital of Medical College, Xi'an Jiaotong University were studied. IFIs were classified as defined by the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group [22]. The exclusion criteria were as follows: (i) age \leq 12 years old, (ii) pregnancy, and (iii) lack of compliance with the recommended dosage regimen.

Some of the characteristics potentially influencing the plasma voriconazole concentration were considered, including age, body weight, liver function, renal function, concomitant medications (inhibitors or inducers of CYP2C19, CYP2C9, or CYP3A4), and CYP2C19 genotype status (ultrarapid metabolizer, extensive metabolizer, intermediate metabolizer, or poor metabolizer).

The study protocol was designed in compliance with the principles of the Helsinki Accords, and was reviewed and approved by the local ethical committee. A statement of informed consent was obtained from all participants after the procedure had been explained to them in full.

Genotyping assay

The blood samples were collected into tubes containing ethylenediaminetetraacetic acid. After centrifugation, the samples were stored at -80°C until analysis. The standard phenol–chloroform extraction method was used to extract genomic DNA from whole blood. DNA concentration was measured by spectrometry (DU-530 UV/Vis spectrophotometer, Beckman Instruments, Fullerton, CA, USA). Twenty-two SNPs (rs1799853, rs1057910, rs12772884, rs1934968, rs9332146, and rs1934967 in CYP2C9; rs2740574, rs4986910, rs4986908, rs4986909, rs12721634, rs4986907, rs4987161, rs4986913, rs4646440, rs4646437, and rs2246709 in CYP3A4; and rs776746, rs10264272, rs41303343, rs4646450, and rs3800959 in CYP3A5) that captured most of the known common CYP2C9, CYP3A4, and CYP3A5 variations were selected for the present study. We also tested the genotype status of CYP2C19 (genotype for *2, *3, and *17) to determine the metabolic types. Sequenom MassARRAY Assay Design 3.0 software was used to design a multiplexed SNP MassEXTEND assay [23–25]. SNPs genotyping was performed using the Sequenom MassARRAY RS1000 system according to the standard protocol recommended by the manufacturer [25]. The primers used for each SNP in the present study are listed in Table 1. Sequenom TYPER 4.0 software was used for data management and analyses [23–26].

Blood sampling and analytical assays

In total, 452 blood samples were collected from the 158 patients receiving voriconazole therapy. The plasma concentrations of voriconazole were determined using the high-performance liquid chromatography (HPLC) method. In brief, a 0.5-mL aliquot of each plasma sample was mixed with 20 μL of internal standard solution (50 mg/L ketoconazole). After vortexing, voriconazole and the internal standard solution were extracted from the plasma using 2 mL of ethyl acetate. The mixture was then centrifuged at 3,000 rpm/min for 10 min, and the supernatant was transferred to a clean culture tube and evaporated to dryness under a stream of nitrogen. The residue was then reconstituted in 100 μL of mobile phase [water (with the addition of 180 mg of ammonium acetate and 80 μL of triethylamine, and adjusted to pH 3.0 using phosphoric acid)/acetonitrile at 60:40, v/v], and a 50- μL aliquot was injected into the HPLC system. Voriconazole and the internal standard were eluted from a C18 column (25 cm \times 4.6 mm; Phenomen, UK) using a mobile phase at a flow rate of 1.0 mL/min and with a run time of 20 min. The standard curve was linear from 0.06 to 8.0 mg/L ($R^2=0.9998$). The intra- and interday precisions were within 6.7 and 7.6 %, respectively [17]. Voriconazole was stable after being stored at room temperature for 24 h, after freezing for 20

Table 1 Primers used for this study

SNP_ID	Allele	1st-PCR	2nd-PCR	UEP_SEQ
rs1799853	C/T	ACGTTGGATGACCCACCCCTTGGTTTTCTC	ACGTTGGATGCTGCGGAATTTTGGGATGGG	AGAGGAGCAITGAGGAC
rs1057910	A/C	ACGTTGGATGAGAGGACAGTGCAGGATCAG	ACGTTGGATGTACACAGATGCTGTGTGTGC	ACGAGGTCCAGAGATAC
rs12772884	A/T	ACGTTGGATGTGGCACAGACAAAAGGAGC	ACGTTGGATGCCCTAACAGAGTTTATAGC	aatgCCTCCTTCTTACTACTCCTT
rs1934968	A/G	ACGTTGGATGGCTCTCCTAGAAAATCTAGC	ACGTTGGATGGAGTTAATAATGTTTTAATTC	tgGGGCTTAAATGTCTCTTTATC
rs9332146	A/G	ACGTTGGATGCAGGATGAACATTTTGGTG	ACGTTGGATGCAACTTCAGTGAGATTCAG	AAATAAAATATGCAAAAATCTGTAG
rs1934967	C/T	ACGTTGGATGGGTATTAGTTTGTCAATTC	ACGTTGGATGAAATTTAGTCAACCCTGCC	cccaCAGAAAATCCAGCCCCAA
rs2740574	A/G	ACGTTGGATGGAAAAC TCAAGTGGAGCCATT	ACGTTGGATGGAATGAGGACAGCCATAGAG	acatCCATAGAGACAAGGGCA
rs4986910	C/T	ACGTTGGATGTGAAGACTCTGATTAGAGC	ACGTTGGATGTTGGAAGTGGACCCAGAAAAC	tggcCCCAGAAAAC TGCATTTGGCA
rs4986908	A/C/G/T	ACGTTGGATGTCAGAACCCCATGGCTGGC	ACGTTGGATGAGAGACAGGCAAGCCTGTCA	GCCTGTACACCTTGAAA
rs4986909	A/C/G/T	ACGTTGGATGAGGAGGGCTCCCTTCCCA	ACGTTGGATGCGTGCACCCAAAAGTACTGGAC	GCCTGAGAAAGTTTCCCTCC
rs12721634	C/T	ACGTTGGATGACTCACAGATAGAGGAGCAC	ACGTTGGATGTGATGGTCTCTCATCCAGAC	tcGAAACCTGGCTTCTCC
rs4986907	A/G	ACGTTGGATGAGATGGTCCCTATCATTTGCC	ACGTTGGATGTGACAGGCTTGCCTGTCT	CCTGTCTCTGCTTCC
rs4987161	C/T	ACGTTGGATGGTGGATTGTTGAGAGATCG	ACGTTGGATGTTCTCCACTCAGCGTCTTTG	TGATCACTAGCACATCAT
rs4986913	C/T	ACGTTGGATGAGAAAATTGACTAACCTGTG	ACGTTGGATGTGCTTAATCAGAGTCCCTC	CTTCAGAACTTCTCCTTCAAA
rs4646440	C/T	ACGTTGGATGCCAACTATGATGTGTGGAGG	ACGTTGGATGGCTAAGGATTTCAAGTCCCTG	CGTCTCGAATGTCTCCC
rs4646437	C/T	ACGTTGGATGCTTCAAAAAGATGCACAAGGG	ACGTTGGATGAGGGCAGGCTATGCATAAG	gTGCATAAGGAGCACC
rs2246709	A/G	ACGTTGGATGCATTTCTATGAAAGTGTCCAG	ACGTTGGATGGCCCTGTCTATCATATGCAACC	gaaCCACTAAATCAACTTTCTG
rs776746	A/G	ACGTTGGATGCCATAATCTCTTTAAAAGAGC	ACGTTGGATGGATGAAAGGGTAAITGGTCC	TCCAAAACAGGGAAGAGATA
rs10264272	C/T	ACGTTGGATGGGACTCTCAACAATCCAC	ACGTTGGATGGGATCTAAGAAACC AAAAT	GAAACCAAATTTTAGGAACTT
rs41303343	-T	ACGTTGGATGCTGTTTTCTTCTTCCAGGC	ACGTTGGATGACCACCATGTCAAGGTACTC	CACGGCATCATAGGTA
rs4646450	C/T	ACGTTGGATGCTAACAAAAGAGCGAGAGGAC	ACGTTGGATGGCTTGTCCAGAAATACACAC	TAATTCACITTCACGTGGCA
rs3800959	C/T	ACGTTGGATGAAATTATGGATGAAGGAAG	ACGTTGGATGAGAGTGTCTCGTAAITTTGCC	AGTCATTTGTCCACTTTTCA
CYP2C19*2	A/C	ACGTTGGATGGCAATAAATTTCCCACTATC	ACGTTGGATGCACATTTCCATAAAGCAAGG	AGTAATTTGTTATGGGTCC
CYP2C19*3	A/G	ACGTTGGATGAACATCAGGATTTGTAAGCAC	ACGTTGGATGGACTGTAAAGTGGTTTTCTCAG	tggggACTTTGGCCCTTACCTGGAT
CYP2C19*17	A/C/T	ACGTTGGATGTGAGCTGAGGTTCTTCTGTATG	ACGTTGGATGCAAAATTTGTGTCTTCTGTTC	aagTTTTGTGTCTTCTGTCTTCAAAAG

or 40 days, and after three freeze–thaw cycles. The patients were divided into three groups according to their average plasma voriconazole concentration: low (<1 mg/L), normal (1–4 mg/L), and high (>4 mg/L).

Statistical analysis

The statistical analyses were performed using SPSS 18.0 for Windows (PASW Statistics, SPSS, Chicago, IL, USA). Differences in genotype and allele frequencies between the low and normal plasma concentration groups and between the high and normal plasma concentration groups were assessed using the χ^2 test. Bonferroni correction for those multiple comparisons was applied to genotype and allele distribution analysis at the $\alpha=0.025$ level. In addition, the association between SNPs and plasma voriconazole concentration was assessed using logistic regression while considering the aforementioned potential confounding factors. The cutoff for statistical significance was set at $p<0.05$ (two-sided).

Results

In total, 158 consecutive patients were included; their characteristics are listed in Table 2. Overall, 40, 91, and 27 patients exhibited low, normal, and high plasma voriconazole concentrations, respectively. Analysis of the genotyping assay results revealed that the following alleles were either not observed in the study population or the minor allele frequencies (MAF) were particularly low: rs1799853 (*2) and rs1057910 (*3) of *CYP2C9*; rs2740574 (*1B), rs4986910 (*3), rs4986908 (*10), rs4986909 (*13), rs12721634 (*14), rs4986907 (*15A), rs4987161 (*17), and rs4986913 (*19) of *CYP3A4*; and rs10264272 (*6) and rs41303343 (*7) of *CYP3A5* (MAF <0.01; data not shown). These SNPs were, therefore, not studied further. The genotype distribution and allele frequencies of the other SNPs in the three plasma voriconazole concentration groups are given in Tables 3 and 4. Only rs4646437 was associated with the plasma voriconazole concentration, and the T allele was associated with a significant predisposition to a high plasma voriconazole concentration ($p=0.019$). After adjustment for the confounding factors associated with the voriconazole concentration, the T allele was still associated with a higher plasma concentration [$p=0.033$, odds ratio (OR)=2.832, 95 % confidence interval (CI)=1.086–7.384].

Discussion

The primary purpose of the present study was to determine the impact of *CYP2C9*, *CYP3A5*, and *CYP3A4* polymorphisms on

the plasma voriconazole concentration, with a view to further clarifying additional factors affecting voriconazole pharmacokinetics in a Chinese Han population. After adjustment for confounding factors, such as age, body weight, liver function, renal function, concomitant medications, and metabolic type, it was discovered that rs4646437 of the *CYP3A4* gene significantly and independently affected the average plasma concentrations of voriconazole, and that the T-variant allele was related to a higher plasma concentration.

CYP2C9 also plays a role in voriconazole metabolism. While there are few published controlled clinical trials regarding the influence of different *CYP2C9* genotypes on the pharmacokinetics and metabolism of voriconazole, there have been some indications that the *CYP2C9* genotype might not be relevant to voriconazole pharmacokinetics. One study found no effect of the *CYP2C9* genotype on voriconazole levels [27]. Furthermore, a case report found that the voriconazole pharmacokinetics of a patient who was homozygous for *CYP2C9*2* were unchanged [18]. In addition, a multiple regression analysis of voriconazole pharmacokinetics found no influence of *CYP2C9* among the 35 healthy study participants [13]. In the present study, we found that the minor allele frequency of *CYP2C9*2* and *CYP2C9*3* SNPs in our population was very low, in accordance with that seen in the Chinese Han population through the HapMap database. This can also be interpreted as the *2 and *3 SNPs not affecting the plasma voriconazole concentration in the Chinese population, in accordance with the findings of the three aforementioned studies [13, 18, 27]. No effect was found for the other SNPs of *CYP2C9*.

The rs776746 (*3) SNP was the most important for the *CYP3A5* gene, which was found in all ethnic groups [28, 29]. Studies of the influence of *CYP3A5* on voriconazole pharmacokinetics have yielded contradictory findings. Weiss et al. found no correlation between the *CYP3A5*3* variant and voriconazole pharmacokinetics [13], and Levin et al. regarded the serum concentrations of a hepatic drug metabolizing enzyme to be a marker of liver toxicity induced by high voriconazole plasma concentrations and found that liver toxicity was not associated with the *CYP3A5*3* allele [19]. However, in vitro studies have shown that voriconazole causes an approximately three-fold higher increase in area under the plasma concentration time curve in individuals with *CYP3A5*3/*3* compared to those expressing at least one functional allele [21]. In the present study, the frequency of the rs776746 GA genotype was higher in the low concentration group than in the normal concentration group, and the probability value was close to reaching marginal significance ($p=0.028$). However, it is important to note that the sample was small, and, so, the effect of this SNP needs to be verified in large samples.

Voriconazole N-oxidation and 4-hydroxylation are catalyzed more efficiently by *CYP3A4* than by *CYP3A5* [30].

Table 2 Characteristics of the 158 patients included in the study

Characteristics	Category	Number
Total		158
Median age (range)		59.35±20.80 (13–99)
Median weight (range)		58.92±8.13 (35–80)
Sex	Male	104 (65.82 %)
	Female	54 (34.18 %)
Administration route	Oral	62 (39.24 %)
	Intravenous	67 (42.41 %)
	Intravenous and oral	29 (18.35 %)
Reason for hospitalization	Pulmonary disease	80 (50.63 %)
	Leukemia	26 (16.46 %)
	Transplantation	8 (5.06 %)
	Other	44 (27.85 %)
Reason for use of voriconazole	Possible IA	23 (14.56 %)
	Probable IA	36 (22.78 %)
	Proven IA	31 (19.62 %)
	Invasive <i>Candida</i>	23 (14.56 %)
	<i>Cryptococcus neoformans</i>	2 (1.27 %)
	Other	43 (27.22 %)
Liver function before voriconazole treatment	Normal	126 (79.75 %)
	Abnormal	32 (20.25 %)
Renal function before voriconazole treatment	Normal	86 (54.43 %)
	Abnormal	72 (45.57 %)
Concomitant liver enzyme inducers	Yes	113 (71.52 %)
	No	45 (28.48 %)
Concomitant liver enzyme inhibitors	Yes	114 (72.15 %)
	No	44 (27.85 %)
Voriconazole metabolic type	Ultrarapid metabolizer	3 (1.90 %)
	Extensive metabolizer	64 (40.51 %)
	Intermediate metabolizer	72 (45.56 %)
	Poor metabolizer	19 (12.03 %)
Voriconazole plasma concentration	Lower	40 (25.32 %)
	Normal	91 (57.59 %)
	Higher	27 (17.09 %)

IA invasive aspergillosis

Numerous variants of *CYP3A4* have been described, but none of them can be held accountable for the high phenotypic variation observed [8]. In the present study, we found that the mutation allele frequencies of the known functional SNPs of *CYP3A4* were very low, in accordance with that seen in the Chinese Han population through the HapMap database. Therefore, these known functional SNPs do not appear to influence voriconazole metabolism in Chinese people. We also studied another three SNPs located in the intron area. Their mutant allele frequencies in our population were high. We found that the rs4646437 mutant allele was associated with a high plasma voriconazole concentration, which represents a novel finding. The influence of this SNP on the plasma concentrations of risperidone and metabolite 9-hydroxy-risperidone, and adverse effects have been studied, but no

significant difference was found [31]. This conclusion was not surprising, since risperidone is metabolized mainly by CYP2D6 [32]. Another study found that the rs4646437 C>T polymorphism could potentially affect the interindividual variability in tacrolimus metabolism among Chinese renal transplant recipients [33]. Tacrolimus is metabolized mainly by CYP3A4 and CYP3A5 [34]. This finding is consistent with the present results. Therefore, although this SNP occurs in an intron, it may affect drugs that are metabolized via CYP3A.

The rs4646437 allele frequency differed significantly between the normal and high plasma voriconazole concentration groups, and the genotype distributions were close to differing significantly. Since the number of mutant homozygotes was small in our studied population, it was difficult to judge whether there was a linear relationship between the number

Table 3 Genotype distributions of ten single-nucleotide polymorphisms (SNPs) in the three concentration groups

SNPs	Genotype	Lower concentration	Normal concentration	Higher concentration	<i>p</i> -Value ^a	<i>p</i> -Value ^b
rs12772884	AA	13 (33.33 %)	29 (31.87 %)	12 (44.44 %)	Ref	Ref
	AT	19 (48.72 %)	45 (49.45 %)	9 (33.33 %)	0.890	0.143
	TT	7 (17.95 %)	17 (18.68 %)	6 (22.22 %)	0.879	0.786
rs1934968	AA	5 (13.16 %)	17 (19.77 %)	4 (15.38 %)	Ref	Ref
	AG	19 (50 %)	39 (45.35 %)	11 (42.31 %)	0.382	0.781
	GG	14 (36.84 %)	30 (34.88 %)	11 (42.31 %)	0.442	0.498
rs9332146	GG	38 (95 %)	88 (96.70 %)	27 (100 %)	Ref	Ref
	AG	2 (5 %)	3 (3.30 %)	0	0.979 ^c	NA
	AA	0	0	0		
rs1934967	CC	26 (66.67 %)	66 (72.53 %)	17 (62.96 %)	Ref	Ref
	CT	13 (33.33 %)	22 (24.18 %)	9 (33.33 %)	0.501 ^c	0.339 ^c
	TT	0	3 (3.30)	1 (3.70 %)		
rs4646440	CC	19 (47.5 %)	61 (67.03 %)	13 (48.15 %)	Ref	Ref
	CT	20 (50 %)	25 (27.47 %)	13 (48.15 %)	0.035 ^c	0.075 ^c
	TT	1 (2.5 %)	5 (5.49 %)	1 (3.70 %)		
rs4646437	CC	30 (75 %)	75 (82.42 %)	17 (62.96 %)	Ref	Ref
	CT	10 (25 %)	16 (17.58 %)	9 (33.33 %)	0.327 ^c	0.032 ^c
	TT	0	0	1 (3.70 %)		
rs2246709	AA	17 (42.5 %)	37 (40.66 %)	9 (33.33 %)	Ref	Ref
	AG	18 (45 %)	43 (47.25 %)	13 (48.15 %)	0.818	0.656
	GG	5 (12.5 %)	11 (12.09 %)	5 (18.52 %)	0.986	0.336
rs776746	GG	14 (35 %)	50 (54.95 %)	14 (51.85 %)	Ref	Ref
	GA	23 (57.5 %)	34 (37.36 %)	10 (37.04 %)	0.028	0.917
	AA	3 (7.5 %)	7 (7.69 %)	3 (11.11 %)	0.870	0.870
rs4646450	CC	14 (35 %)	49 (53.85 %)	14 (51.85 %)	Ref	Ref
	CT	23 (57.5 %)	35 (38.46 %)	10 (37.04 %)	0.038	1
	TT	3 (7.5 %)	7 (7.69 %)	3 (11.11 %)	0.890	0.890
rs3800959	TT	28 (71.79 %)	71 (78.02 %)	19 (70.37 %)	Ref	Ref
	CT	11 (28.21 %)	18 (19.78 %)	7 (25.93 %)	0.445 ^c	0.412 ^c
	CC	0	2 (2.20 %)	1 (3.70 %)		

NA not available

^a *p*-Values were calculated from the two-sided χ^2 test; genotype frequency in the lower concentration group vs. in the normal concentration group

^b *p*-Values were calculated from the two-sided χ^2 test; genotype frequency in the higher concentration group vs. in the normal concentration group

^c Considering heterozygotes and homozygotes together

of mutant alleles being carried and the voriconazole concentration. Research involving a large number of samples is needed in order to confirm this kind of contact. We used logistic regression while correcting for some potentially confounding factors to further confirm the association between alleles and the plasma voriconazole concentration. The association between the T allele and a high plasma voriconazole concentration persisted after applying logistic regression.

Voriconazole has been widely used clinically. Since it is metabolized mainly via CYP450, many factors could affect its metabolic processes in the body, among which the CYP450 gene mutation is an important factor. There have been many investigations of how CYP450 gene SNPs influence

voriconazole efficacy, both before and after this drug was introduced into the clinical setting, although most of these studies have focused on SNPs in exons and promoters. In the present study, the significant SNPs were located in the intron area, and, therefore, did not (in theory) induce a change in the encoding protein. However, Choi et al. indicated that it is possible for an intronic SNP to alter the splicing of primary transcripts or gene expression [35]. Many other studies have also revealed that specific subsets of exonic splicing silencers exert distinct effects on a multifunctional intron retention reporter, and that one of these subsets is the most likely to be involved in the regulation of endogenous intron retention events. Intron retention could, therefore, affect alternative

Table 4 Allele frequencies of ten single-nucleotide polymorphisms (SNPs) in the three concentration groups

SNPs	Allele	Lower concentration	Normal concentration	Higher concentration	<i>p</i> -Value ^a	<i>p</i> -Value ^b
rs12772884	A	45 (57.69 %)	103 (56.59 %)	33 (61.11 %)	0.870	0.555
	T	33 (42.31 %)	79 (43.41 %)	21 (38.89 %)		
rs1934968	A	29 (38.16 %)	73 (42.44 %)	19 (36.54 %)	0.527	0.448
	G	47 (61.84 %)	99 (57.56 %)	33 (63.46 %)		
rs9332146	G	78 (97.5 %)	179 (98.35 %)	54 (100 %)	0.979	NA
	A	2 (2.5 %)	3 (1.65 %)	0		
rs1934967	C	65 (83.33 %)	154 (84.62 %)	43 (79.63 %)	0.795	0.386
	T	13 (16.67 %)	28 (15.38 %)	11 (20.37 %)		
rs4646440	C	58 (72.5 %)	147 (80.77 %)	39 (72.22 %)	0.135	0.177
	T	22 (27.5 %)	35 (19.23 %)	15 (27.78 %)		
rs4646437	C	70 (87.5 %)	166 (91.21 %)	43 (79.63 %)	0.355	0.019
	T	10 (12.5 %)	16 (8.79 %)	11 (20.37 %)		
rs2246709	A	52 (65 %)	117 (64.29 %)	31 (57.41 %)	0.911	0.359
	G	28 (35 %)	65 (35.71 %)	23 (42.59 %)		
rs776746	G	51 (63.75 %)	134 (73.63 %)	38 (70.37 %)	0.106	0.636
	A	29 (36.25 %)	48 (26.37 %)	16 (29.63 %)		
rs4646450	C	51 (63.75 %)	133 (73.08 %)	38 (70.37 %)	0.128	0.696
	T	29 (36.25 %)	49 (26.92 %)	16 (29.63 %)		
rs3800959	T	67 (85.90 %)	160 (87.91 %)	45 (83.33 %)	0.655	0.382
	C	11 (14.10 %)	22 (12.09 %)	9 (16.67 %)		

NA not available

^a *p*-Values were calculated from the two-sided χ^2 test; allele frequency in the lower concentration group vs. in the normal concentration group

^b *p*-Values were calculated from the two-sided χ^2 test; allele frequency in the higher concentration group vs. in the normal concentration group

splicing of pre-mRNA by influencing the exonic splicing silencers, thus producing different mature mRNAs and then translating proteins with different functions [36–38]. Therefore, in the present study, we selected SNPs associated with the voriconazole concentration from the statistical perspective; these SNPs may also have an impact on protein function. Further studies are needed in order to ascertain the biological function of the proteins encoded by these SNPs.

The smallness of the sample was the main limitation of this study. Some homozygous mutations did not appear, and, thus, it was not possible to determine the real genotype distribution. The limitation of the small sample also made it difficult to determine whether there was a linear relationship between gene mutation and the plasma voriconazole concentration. In addition, due to technical reasons, some SNPs were not genotyped successfully in several patients.

In summary, this study provides preliminary evidence that rs14646437 could be a factor underlying a higher plasma voriconazole concentration, suggesting that more data are needed to verify the correct voriconazole dosing in patients. But it needs to be confirmed using large-scale data validation, and more research is needed into the mechanism underlying the function of this SNP.

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Conflict of interest The authors declare that they have no conflict of interest.

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