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Relationship of Cytochrome P450 gene polymorphisms with blood concentration of hydroxychloroquine and its metabolites and adverse drug reactions

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ABSTRACT

Background: Hydroxychloroquine (HCQ) is a cornerstone therapy for systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). This study aimed to investigate the relationship of cytochrome P450 (CYP450) gene polymorphisms with blood concentration of HCQ and its metabolites and adverse drug reactions (ADRs) in patients with SLE and RA.

Methods: A cohort of 146 patients with SLE and RA treated with HCQ was reviewed. The ADRs of patients were recorded. The blood concentration of HCQ and its metabolites were measured by liquid chromatography–mass spectrometry analysis. Genotyping of single nucleotide polymorphism (SNP) in CYP450 metabolic enzyme involved in HCQ metabolic pathway was performed using a MassARRAY system. Chi-square test, T-test, and one-way analysis of variance were used to analyze data.

Results: Among 29 candidate SNPs, we found that CYP3A4 (rs3735451) was significantly associated with blood levels of HCQ and its metabolites in unadjusted model and adjusted model (patients taking HCQ for >10 years) ($P<0.05$). For CYP3A5 (rs776746), skin and mucous membrane ADRs associated with the TT genotype were a greater risk than for the CT+CC genotypes ($P=0.033$). For CYP2C8 (rs1058932), abnormal renal function with the AG genotype carried a greater risk than with the AA+GG genotype ($P=0.017$); for rs10882526, ophthalmic ADRs of the GG genotype carried a greater risk than for the AA+AG genotypes ($P=0.026$).

Conclusions: The CYP2C8 (rs1058932 and rs10882526) and CYP3A5 (rs776746) polymorphisms are likely involved in the ADRs of HCQ. Gene polymorphism analysis of CYP450 and therapeutic drug monitoring of HCQ and its metabolites might be useful to optimize HCQ administration and predict ADRs.

Keywords: Hydroxychloroquine, CYP450 gene polymorphism, Blood concentration, Adverse reactions, Systemic lupus erythematosus, Rheumatoid arthritis

INTRODUCTION

Autoimmune diseases (AIDs) such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) are caused by immune tolerance deficiency or abnormal immune regulation and lead to damage of host organs [1]. Glucocorticoids (GCs) and disease-modifying anti-rheumatic drugs (DMARDs) are routinely prescribed drugs, which have shown good therapeutic effects on disease control [2]. Currently, non-biological DMARDs like azathioprine (AZA), methotrexate (MTX), and hydroxychloroquine (HCQ) play a role in relieving pain and inhibiting disease progression [3]. Among these drugs, HCQ was initially used as an antimalarial medication and then translated to rheumatic diseases. Currently, HCQ is the mainstay treatment for SLE; according to the latest European guidelines, it is recommended for all SLE patients unless contraindicated or with adverse effects [4]. The latest European League Against Rheumatism (EULAR) recommendations stated that HCQ was also part of the triple therapy in RA, and HCQ was combined to methotrexate and sulfasalazine to increase the response rate in RA [5]. Despite the efficacy of HCQ in treating manifestations of SLE and RA, common side effects such as headaches, dizziness, gastrointestinal symptoms, and rash have been reported [6]. Notably, retinopathy is a serious side effect of HCQ, and regular ophthalmologic monitoring is recommended for patients on long-term HCQ therapy [7, 8].

Hydroxychloroquine shows large interindividual variations in its concentration, despite individuals taking the same dose. For SLE patients on long-term oral HCQ treatment, a lower the SLE disease activity index (SLEDAI) score was significantly related to higher blood HCQ concentration [9].

Moreover, metabolites of HCQ such as desethyl hydroxychloroquine (DHCQ) and desethyl chloroquine (DCQ) exhibited a concentration-effect relationship in patients with RA; bisdesethyl chloroquine (BDCQ) has also been implicated in HCQ toxicity [10]. HCQ is metabolized into DHCQ, DCQ, and BDCQ by cytochrome P450 enzymes (CYP450s) 3A4/5, 2C8, and 2D6 in vivo by *N*-deethylation [11], in which DHCQ is the major metabolite and the activated form of HCQ. The CYP 2D6*10 (rs1065852) polymorphism was significantly related to the level of DHCQ, while CYP3A5*3 (rs776746) and CYP 3A4*18 (rs28371759) did not show any significant association with the levels of HCQ and DHCQ [12]. However, to our knowledge, few studies thus far have reported the relationship between the adverse drug reactions (ADRs) of HCQ and CYP450 polymorphisms [13]. Understanding this relationship will be helpful to refine HCQ dosage. Therefore, we analyzed the influence of CYP450 gene polymorphisms, mainly involving the 2D6, 3A4, 3A5, and 2C8 polymorphism, on the blood concentration of HCQ and its metabolites, as well as the risk of ADRs.

PATIENTS AND METHODS

Study design and population. This was a prospective, observational, single-center clinical trial designed to examine the influence of CYP450 gene polymorphisms on the blood concentration and ADRs of HCQ and its metabolites. The study subjects were from the Outpatient Department of Rheumatology and Immunology at the First Affiliated Hospital of Anhui Medical University and who had been taking long-term oral HCQ. The patients' general and clinical details were recorded in detail. The inclusion criteria for SLE and RA was as follows: patients met either the 2010 RA classification criteria defined by the American College of Rheumatology (ACR) and the EULAR, and EULAR/ACR-2019 for SLE [14,15]; received treatment with oral HCQ for >6 months; were on a daily

dosage of 200–400 mg; and consented to donate blood samples for the study. The exclusion criteria were as follows: non-SLE and non-RA patients; pregnant and lactating women; patients with incomplete data; patients with poor compliance; and those with renal impairments, eye disease, or cutaneous damage before receiving HCQ. Blood samples (10mL) were collected during an outpatient visit for determining HCQ, DHCQ, DCQ, and BDCQ blood concentrations and genetic testing. The following laboratory tests were conducted: complete blood cell count, erythrocyte sedimentation rate, C-reactive protein level, levels of C3 and C4, and anti-double-stranded DNA antibody titer.

Liquid chromatography–mass spectrometry (LC-MS/MS) analysis of HCQ, DHCQ, DCQ, and BDCQ blood levels. We measured the blood concentrations of HCQ, DHCQ, DCQ, and BDCQ at Anhui Medical University Scientific Research Experiment Center (Anhui Medical University, Hefei City, China) with LC-MS/MS, according to the method of Chhonker et al. [16], by using an AB Sciex 5500 LC-30AD pump SIL-30AC autosampler (AB SCIEX, Los Angeles, CA, USA). The analytes were separated on Poroshell 120 EC-C18 (2.1×100 mm, 2.7-μm thickness, Agilent Technologies, CA, USA), at a column temperature of 35°C and flow rate of 0.25 mL/min. The internal standard for the LC-MS/MS assay was chloroquine (CQ). The MS data of blood separated by the above-mentioned optimized high-performance liquid chromatography (HPLC) method were processed by the Analyst 1.6.3 data processing software system (AB SCIEX, Los Angeles, CA, USA). According to the internal standard method, the standard curve was established to calculate the blood levels of HCQ, DHCQ, DCQ, and BDCQ.

Evaluation of ADRs. The ADR evaluation form for SLE and RA patients on HCQ were made according to the World Health Organization–Uppsala Monitoring Centre (WHO-UMC) system [17]

and recorded in our study. Patients with serious side effects were assigned to a specialist clinic for further treatment and regular follow-up until symptoms were stable.

DNA extraction and genotyping of CYP SNPs. Genomic DNA was extracted using a commercially available DNA extraction kit (Tiangen Biotech, Beijing, China) according to the manufacturer's instructions. The extracted genomic DNA was then stored at -20°C until analysis.

Polymerase chain reaction (PCR) primers and single-base extension primers were designed according to the Assay Design Suite V2.0 (Sequenom) online software (http://www.mysequenom.com). In this study, a total of 29 SNPs from four CYP450s were selected for genetic polymorphism analysis: CYP3A4 (rs28371759, rs4646440, rs4646437, rs3735451, rs2246709, and rs2242480); CYP3A5 (rs1419745, rs4646450, rs15524, rs776746, and rs3800959); CYP2C8 (rs2071426, rs17110453, rs1341159, rs1557044, rs10772526, rs6583969, rs11572139, rs7909236, rs2185571, rs1934952, rs11572162, and rs1058932); and CYP2D6 (rs28371699, rs4078247, rs28670611, rs1080983, rs35028622, and rs5758589). SNP typing was completed on a MassARRAY system (Sequenom), which was based on matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF). The PCR amplification conditions were as follows: pre-denaturation at 95°C for 5 min; followed by 25 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 30 s, extension at 72°C for 1 min; and a final extension at 72°C for 5 min. The final results were genotyped by MassARRAY Typer 4.0 software system (Sequenom Inc., San Diego, CA, USA).

Statistical analysis. Data were analyzed using IBM SPSS Statistics (version 26, IBM Corporation, Armonk, NY, USA). Quantitative data were presented as mean and standard deviation, while qualitative data were presented as number and percentage. Chi-square test was used to compare Hardy–Weinberg equilibrium and the adverse reactions of patients with different genotypes, and T-test

and one-way analysis of variance were used to compare the blood concentration of HCQ among groups. $P<0.05$ was considered to indicate statistical significance.

RESULTS

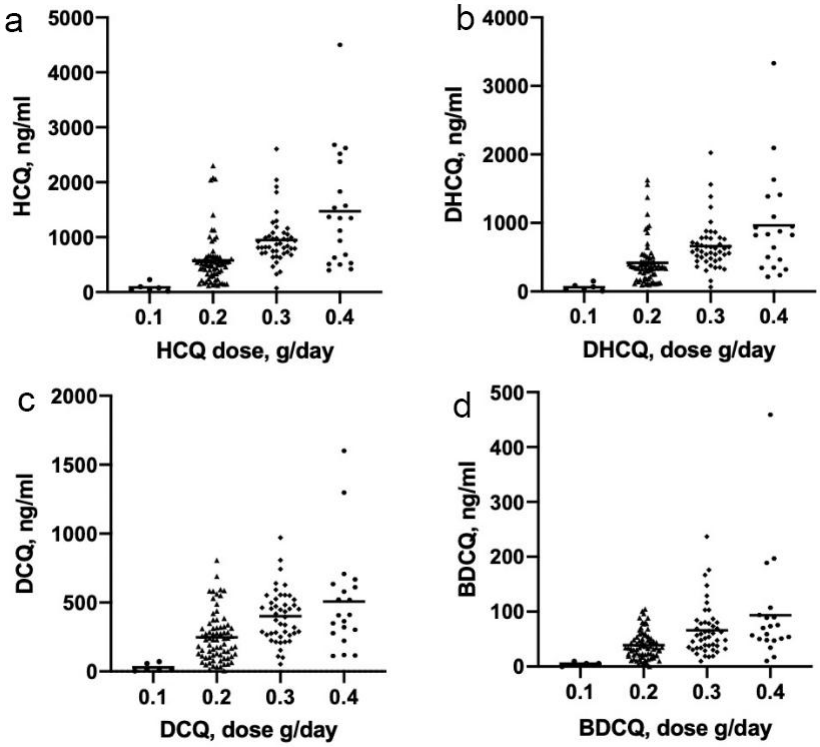
Patient characteristics. A total of 146 participants (n=121 SLE and n=25 RA; seven male and 139 female; mean age: 42.27±14.13 years) with pathologic diagnosis were enrolled. The characteristics of the patients are shown in **Table 1**. The mean duration of oral HCQ was 51.70±46.50 months, and the mean daily dose of HCQ was 255.48±77.93 mg/day. The mean disease activity score 28(DSA28) score of RA patients was 2.16±0.41, and the mean SLEDAI score of SLE patients was 0.89±1.81, which suggested that most participants had mild disease (DSA28<3.2 and SLEDAI<9). The plasma concentrations of HCQ, DHCQ, DCQ, and BDCQ were 838.10±522.00, 582.80±363.90, 346.90±205.30, and 56.00±39.30 ng/ml, respectively. Grouped according to the dosage of patients, the results showed that blood concentrations of HCQ, DHCQ, DCQ, and BDCQ were significantly related to the daily HCQ dosage ($P<0.005$) (**Fig. 1)**. The relationships between blood concentrations of HCQ, DHCQ, DCQ, and BDCQ and the duration of oral HCQ are shown in **Fig. 2a–2d**, respectively. The blood concentration of HCQ and the blood concentration of DHCQ, DCQ, and BDCQ also had a significant correlation ($P<0.005$). The distribution of ADRs among all patients was as follows: abnormal renal function (n=21), abnormal liver function (n=11), ophthalmic ADRs (n=20), and skin and mucous membrane ADRs (n=15).

Table 1 Characteristics of the study participants

Characteristic	No. of patients, N=146
SLE, no. (%)	121(82.80)
RA, no. (%)	25(17.20)
Age, mean±SD years	42.27±14.13
Female, no. (%)	139(95.20)
Weight, mean±SD kg	56.89±9.21
Duration of HCQ treatment, mean±SD months	51.70±46.50
HCQ dose, mean±SD mg/day	255.48±77.93
HCQ dose, mean±SD mg/kg/day	4.58±1.50
Daily prednisolone dose, mean±SD mg	4.77±4.33
DSA28 score, mean±SD	2.16±0.41
SLEDAI score, mean±SD	0.89±1.81
[HCQ], mean±SD ng/ml	838.10±522.00
[DHCQ], mean±SD ng/ml	582.80±363.90
[DCQ], mean±SD ng/ml	346.90±205.30
[BDCQ], mean±SD ng/ml	56.00±39.30
ADR, no. (%)	
Abnormal renal function	21(14.30)
Abnormal liver function	11(7.50)
Ophthalmic ADRs	20(13.70)
Skin and mucous membrane ADRs	15(10.30)

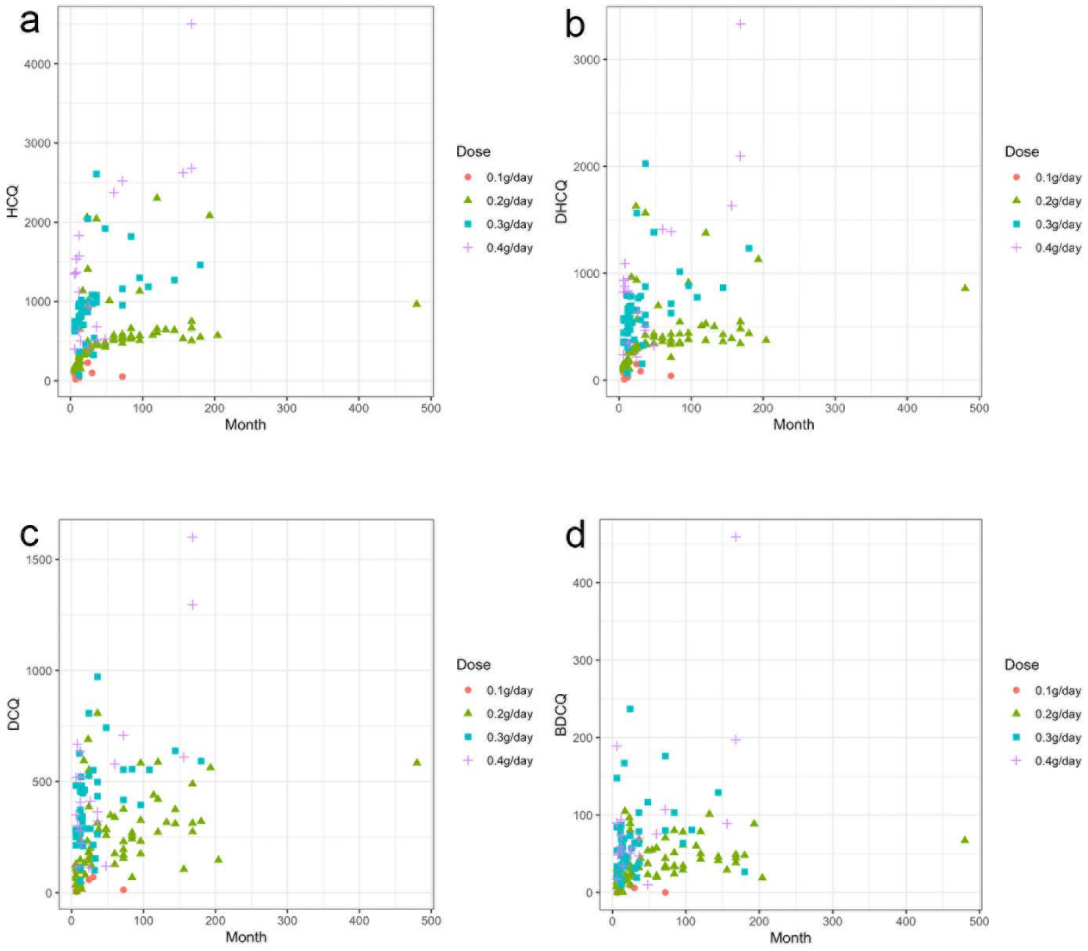
SLEDAI=Systemic Lupus Erythematosus Disease Activity Index; ADRs=Adverse drug reactions; Disease activity score 28=DAS28; [HCQ]=HCQ concentration; [DHCQ]=Desethyl hydroxychloroquine concentration; [DCQ]=Desethyl chloroquine concentration; [BDCQ]=Bisdesethyl chloroquine concentration

Fig. 1 Correlation between the daily dose groups and concentration of HCQ and its metabolites.



a, HCQ; b, DHCQ; c, DCQ; and d, BDCQ.

Fig. 2 Time-course of blood concentrations of HCQ and its metabolites in SLE and RA patients receiving 100, 200, 300, or 400 mg HCQ daily.



a, HCQ; b, DHCQ; c, DCQ; and d, BDCQ (n=6, n=74, n=46, and n=20 for 100, 200, 300, and 400 mg dose groups, respectively.)

Hardy–Weinberg equilibrium (HWE). The genotype distribution based on HWE is shown in **Table 2**. The genotyping results of the 29 SNPs in 146 SLE and RA patients showed that, except for CYP 2C8 (rs1341159) and CYP2D6 (rs28371699, rs4078247, rs35028622, rs28670611, and rs1080983), no other mutations were detected; therefore, no statistical analysis was required. The allele frequencies of the remaining 23 SNPs were consistent with HWE (P>0.05), and the observed and expected values of alleles and genotypes showed good agreement, which indicated that the samples included in this study were representative of the population.

Table 2 HWE test of genotypes in 146 patients

Polymorphism	SNP	HWE P-value	Polymorphism	SNP	HWE P-value
CYP 2C8	rs2071426	0.23758	CYP3A4	rs28371759	0.93359
	rs17110453	0.09269		rs4646440	0.29517
	rs1341159	0.04798*		rs4646437	0.75722
	rs1557044	0.18561		rs3735451	0.85980
	rs10882526	0.69981		rs2246709	0.54361
	rs6583969	0.39241		rs2242480	0.12091
	rs11572139	0.40038	CYP2D6	rs28371699	0.00160*
	rs7909236	0.34911		rs4078247	0.00947*
	rs2185571	0.40038		rs28670611	0.00235*
	rs1934952	0.25790		rs1080983	0.00144*
	rs11572162	0.54993		rs35028622	0.00147*
	rs1058932	0.79740		rs5758589	0.07308
CYP3A5	rs1419745	0.91067			
	rs4646450	0.95620			
	rs15524	0.61950			
	rs776746	0.77629			
	rs3800959	0.43860			

*P<0.05, means no genetic mutation, and no statistical analysis is needed.

Relationship between CYP450 gene polymorphisms and ADRs. Samples from 146 patients with SLE and RA were used to analyze the association of SNPs with the risk of ADRs. In the genotype distribution analysis, CYP2C8 (rs1058932 and rs10882526), CYP3A5 (rs776746), and CYP3A4 (rs3735451) were related to ADRs. The frequencies of these polymorphisms are shown in **Table 3**. The ADRs of patients with CYP2C8 (rs1058932), CYP2C8 (rs10882526) and CYP3A5 (rs776746) genotype are shown in **Table 4**. Only 21 patients with abnormal renal function were involved in the analysis of SNPs. Abnormal renal function with the AG genotype was greater than with the AA genotype and GG genotype of CYP2C8 (rs1058932) (P=0.017), while no statistical difference in the different genotypes of CYP3A4 and CYP3A5 were noted (P>0.05). The CYP2C8 (rs10882526) is related to ophthalmic ADRs, the risk of the AA genotype was greater than that of the GG and AG genotypes (P=0.026), while different genotypes of CYP3A4 and CYP3A5 showed no significant difference between normal and abnormal groups (P>0.05). CYP3A5 (rs776746) was related with the incidence of skin and mucous membrane ADRs in our research (P=0.033), and the risk of the TT genotype was greater than that of the CC and CT genotypes; no significant difference was noted between the normal and abnormal skin and mucous membrane ADRs in different genotypes of CYP3A4 and CYP2C8 (P>0.05). For the incidence rate of abnormal liver function, there was no significant difference in selected SNPs (P>0.05).

Table 3 Frequencies of CYP 2C8, 3A5, and 3A4 polymorphisms

	Oligonucleotide primer	Allele frequency	Genotype	n (%)	HWE P-value
CYP2C8 (rs1058932)	r 5'-CTAGCCCATCTGGCTGC-3'	A=35.3%	A/A	23(15.8)	0.79740
		G=64.7%	A/G	57(39.0)	
			G/G	66(45.2)	
CYP2C8 (rs10882526)	f 5'-TCAACTCACTCCGCT-3'	A=87.0%	G/G	3(2.1)	0.69981
		G=13.0%	A/A	111(76)	
			A/G	32(21.9)	
CYP3A5 (rs776746)	f 5'-TCCAAACAGGGAAGAGATA-3'	C=79.1%	C/T	55(37.7)	0.77629
		T=20.9%	T/T	8(5.5)	
			C/C	83(56.8)	
CYP3A4 (rs3735451)	f5'-AACAGAGTGATATTCTGATCTC-3'	C=26.7%	C/C	10(6.9)	0.85980
		T=73.3%	C/T	58(39.7)	
			T/T	78(53.4)	

Values are the number (%).

Table 4 ADRs of patients with CYP2C8 (rs1058932), CYP2C8 (rs10882526), and CYP3A5 (rs776746)

ADR	Gene	SNP	Genotype	Normal group (n)	Abnormal group (n)	*P*
Renal function	CYP2C8	rs1058932	AA	22	1	0.017
			AG	43	14	
			GG	60	6	
Ophthalmic	CYP2C8	rs10882526	GG	1	2	0.026
			AA	97	14	
			AG	28	4	
Skin and mucous membrane	CYP3A5	rs776746	CT	50	5	0.033
			TT	5	3	
			CC	36	7	

Relationship between CYP450 gene polymorphism and blood concentrations of HCQ and its metabolites. In the unadjusted model (patients taking medication for ≤10 years), only the CYP3A4 (rs3735451) polymorphism showed significant differences with the blood concentrations of HCQ, DCQ, and BDCQ (*P*=0.033, *P*=0.039, and *P*=0.033). The mean blood concentration of HCQ in SLE and RA patients with different CYP3A4 (rs3735451) genotypes are shown in **Table 5**. The HCQ, DCQ, and BDCQ concentrations were higher in patients with CC genotype than with CT genotype. After adjusting for HCQ medication time (patients taking medication for >10 years), this significant correlation still existed. For HCQ, DCQ, and BDCQ, the comparison between CC and CT genotype in

patients was statistically significant (P<0.05). The relationship between the genotypes of CYP3A4 (rs3735451) and the mean blood concentrations of HCQ, DHCQ, and DCQ were TT>CT>CC, while the CC genotype was lower than for the other two genotypes of BDCQ.

Table 5 Results of average blood concentration of HCQ and its metabolites in SLE and RA patients with different CYP3A4 genotypes

	SNP	Group	Genotype	n	Blood concentration (ng/mL)	*P*
Unadjusted model	rs3735451	HCQ	CC	8	742.4±542.6*	0.033
			CT	53	723.8±532.9	
			TT	69	742.1±543.9	
		DHCQ	CC	8	512.3±365.4	0.071
			CT	53	500.1±358.1	
			TT	69	512.1±367.4	
		DCQ	CC	8	298.0±200.3*	0.039
			CT	53	293.3±198.8	
			TT	69	299.5±202.9	
		BDCQ	CC	8	48.9±39.2*	0.033
			CT	53	48.0±38.5	
			TT	69	48.8±39.0	
Adjusted Model[#]	rs3735451	HCQ	CC	10	759.9±569.6*	0.017
			CT	58	795.9±651.9	
			TT	78	797.0±651.9	
		DHCQ	CC	10	519.8±381.6	0.083
			CT	58	553.5±452.8	
			TT	78	554.1±454.0	

DCQ	CC	10	305.6±271.3*	0.047
	CT	58	322.3±239.3	
	TT	78	322.7±241.0	
BDCQ	CC	10	49.7±40.4*	0.005
	CT	58	53.5±52.5	
	TT	78	53.3±52.4	

Note: vs. CT genotype, *P<0.05 #: Adjusted for duration of use.

Discussion

Hydroxychloroquine is a hydroxyl derivative of CQ and similar antimalarial activity as CQ. However, because HCQ has low toxicity, it can be used in the long-term treatment of SLE and RA. The plasma elimination half-life of HCQ is about 40–60 days, with higher tissue distribution and longer residence time *in vivo* [18]. Despite the potential efficacy and relative safety, the indications for and dosage of HCQ should be strictly controlled in clinical treatment. Furthermore, the ADRs should be closely observed, and HCQ blood levels of patients should be measured at regular intervals if necessary [19].

Studies have shown that there is a positive correlation between the efficacy of HCQ and its blood concentration. HCQ shows large inter-individual variations terms of its concentration, despite individuals taking the same dose [10]. About 40% of patients are unresponsive or intolerant to HCQ [20]. HCQ is mainly deacetylated by CYP450 enzymes 2D6, 2C8, and CYP3A4/5 [11,12], therefore the gene polymorphism of HCQ metabolic enzymes may be an important factor that affects individual concentration differences. Lee et al. [12] studied the effect of CYP2D6 gene polymorphisms on the blood concentration of HCQ in patients with SLE. They found a significant correlation between

rs1065852 and rs1135840 and the ratio of DHCQ:HCQ, which indicated that the blood concentration of HCQ was related to CYP2D6 gene polymorphism, but no similar results were found in CYP3A5*3 (rs776746) and CYP3A4*18B (rs28371759). Although some studies have reported that there was no significant correlation between CYP2C8 gene polymorphism and patients' response to HCQ [10], our previous research showed that SLE patients with CYP2C8 (rs10882521) GT genotype who took the same dose of HCQ had lower blood concentration than those with other genotypes, indicating that this SNP is related to the blood concentration of HCQ [21].

CYP3A4 is the most abundant CYP450 enzyme in the human liver, accounting for about 80% of the total CYP450 enzyme. The newly discovered CYP3A4*1G (rs2242480) is the site with the highest mutation frequency in the CYP3A4 allele, and the CYP3A4*1G gene polymorphism can reduce the catalytic activity of the CYP3A4 enzyme [22]. In our present study, a correlation between CYP3A4 (rs28371759) and (rs2242480) gene polymorphism and blood concentration of HCQ was not found, which was consistent with that reported in the literature [12]. We found that CYP3A4 (rs3735451) was significantly correlated with blood concentration of HCQ and its metabolites by adjusting for the time of administration, and the mean blood concentrations of HCQ, DHCQ, and DCQ in patients with CC, CT, and TT genotypes is higher than that of other genotypes, in which the blood concentration of HCQ and its main metabolite DHCQ was the lowest for the CC genotype. It is suggested that for patients with CYP3A4 (rs3735451) site mutation, a higher dose may be needed in clinical treatment to achieve effective blood concentration and therapeutic effect.

Common ADRs are listed in the manual of HCQ sulfate tablets, including vision, skin, gastrointestinal tract, central nervous system, neuromuscular, cardiovascular system, hematology, and liver, and other allergic reactions [6,23]. In fact, Munster et al. [10] found a correlation between gastrointestinal

adverse events and elevated blood HCQ levels, and a potential relationship between ophthalmic adverse events and BDCQ levels. The incidence of ophthalmic ADRs in previous literature was 7.5% [24]; the incidence in our present study was 14.4%, significantly higher than that reported previously reported. This may be due to the inclusion of more ophthalmic ADRs in our study, such as eye swelling, hyperemia, blurred vision, and conscious ametropia. However, until now, there have been only few reports about the association between ADRs of HCQ and CYP450 gene polymorphisms. In this study, we found a significant difference in the distribution of CYP2C8 (rs10882526) GG genotype and AA+AG genotypes in the ophthalmic ADR group ($P<0.05$), and the incidence of ADRs for the GG genotype was higher than that of AA+AG genotypes. The most important predictors of ophthalmic ADRs in patients are high doses and long-term use of HCQ; hence, mandatory ophthalmic evaluation should be performed during long-term use of HCQ to ensure drug safety [25].

HCQ is eventually excreted through the kidney. The reduced clearance in patients with renal insufficiency will lead to HCQ accumulation in the body, significantly increase the blood concentration after medication, and the risk of drug poisoning. However, the dosage of HCQ in patients with renal insufficiency is not clear [26]. In this study, we analyzed the relationship between CYP2C8 gene polymorphism and renal dysfunction. As a result, there was a significant difference in the distribution of AG genotype and AA+GG genotypes of CYP2C8 (rs1058932) between the normal and abnormal renal function groups. In addition, the distribution of CYP3A5 (rs776746) TT genotype and CT+CC genotypes in patients with long-term HCQ use was significantly different from that in the normal group ($P<0.05$). There are few reports about liver injury caused by HCQ; these mainly include liver drug enzyme abnormality and acute liver failure [27, 28]. Abnormal liver function, manifested by liver enzyme increase (aspartate aminotransferase, AST), was found in 11 patients in our study, but no

influence of CYP450 gene polymorphism was found. The possible reason was that we only took the dose and course of treatment into account, and the combined medication was not considered. These results suggest that patients should regularly monitor their liver function during HCQ treatment and should reduce the dose or stop medication if necessary.

An earlier study showed that the DHCQ: HCQ ratio was related to CYP2D6 (rs1065852 and rs1135840) polymorphisms after taking oral HCQ [12]. Our result was not consistent with that previous study, and we could not examine this polymorphism in our study because the frequency of CYP2D6 polymorphisms were confirmed to be extremely low in our study population (based on HWE analysis). Another possible limitation would be the limited sample size in our study. Future studies with larger sample sizes may be useful to investigate any associations between the CYP2D6 SNPs and HCQ metabolism and ADRs.

The gene polymorphism of CYP enzyme may be an important determinant of drug sensitivity and ADRs among different individuals. In the clinic, patients with CYP2C8 (rs1058932) AG genotype had a higher incidence of renal dysfunction than AA genotype and GG genotype after taking HCQ. Patients with CYP2C8 (rs10882526) GG genotype had a higher incidence of ophthalmic ADRs than AA genotype and AG genotypes after taking HCQ. In addition, patients with CYP3A5 (rs776746) TT genotype had a higher incidence of skin and mucosal ADRs than CC genotype and CT genotypes after taking HCQ. Given the same dose of HCQ, the blood concentration of HCQ in patients with CYP3A4 (rs3735451) CC genotype was lower than that in patients with other genotypes. Thus, it may be helpful to improve the efficacy and reduce the ADRs by genotyping the related CYP450 gene polymorphisms before administration of HCQ and monitoring the concentration of HCQ and its metabolites. Physicians should try to offer individualized treatment with HCQ based on the results of CYP2C8

(rs1058932 and rs10882526), CYP3A4 (rs3735451), and CYP3A5 (rs776746) genotypes and the HCQ blood concentration.

Abbreviations

HCQ: Hydroxychloroquine; SLE: Systemic lupus erythematosus; RA: Rheumatoid arthritis; CYP450:Cytochrome P450; ADRs: Adverse drug reactions; SNP: Single nucleotide polymorphism; AIDs: Autoimmune diseases ; GCs: Glucocorticoids; DMARDs: Disease-modifying anti-rheumatic drugs; AZA: Azathioprine; MTX: Methotrexate; SLEDAI: SLE disease activity index; DHCQ: Desethylhydroxychloroquine; DCQ: Desethylchloroquine; LC-MS/MS: Liquid chromatography–mass spectrometry; BDCQ: Bisdesethyl chloroquine; CQ: chloroquine; HPLC: High-performance liquid chromatography; HWE: Hardy–Weinberg equilibrium.

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Authors' contributions

BG, JW, QX designed in the study, participated in data collection, conducted data analysis, and drafted. ZS, TT, and MP participated in data collection, contributed to the analysis and to the analysis. XC and CY performed data analysis. All the authors contributed substantially to the work, revised the manuscript critically, approved the submitted version, and agree to be accountable for all aspects of the work.

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Availability of data and materials

The datasets used and analyzed in this study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate: The study complies with the Research and Ethics Committee of the First Affiliated Hospital of Anhui Medical University has approved the research protocol (reference: PJ2020-16-25) and all methods were performed in accordance with the relevant guidelines and regulations of The Declaration of Helsinki (DoH). Informed written consent had been obtained from all participants in the study.

Consent for publication

All the authors approved the submitted version for publication.

Competing interests

Authors have no conflicts of interest to declare with regard to this work.

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