Parasitology Research (2019) 118:1011–1018
https://doi.org/10.1007/s00436-019-06210-3

TREATMENT AND PROPHYLAXIS - ORIGINAL PAPER

Effects of MAO-A and CYP450 on primaquine metabolism in healthy volunteers

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Received: 14 August 2018 / Accepted: 10 January 2019 / Published online: 31 January 2019

Abstract

Eliminating the *Plasmodium vivax* malaria parasite infection remains challenging. One of the main problems is its capacity to form hypnozoites that potentially lead to recurrent infections. At present, primaquine is the only drug used for the management of hypnozoites. However, the effects of primaquine may differ from one individual to another. The aim of this work is to determine new measures to reduce *P. vivax* recurrence, through primaquine metabolism and host genetics. A genetic study of MAO-A, CYP2D6, CYP1A2 and CYP2C19 and their roles in primaquine metabolism was undertaken of healthy volunteers ($n = 53$). The elimination rate constant (K_e) and the metabolite-to-parent drug concentration ratio (Cm/Cp) were obtained to assess primaquine metabolism. Allelic and genotypic analysis showed that polymorphisms MAO-A (rs6323, 891G>T), CYP2D6 (rs1065852, 100C>T) and CYP2C19 (rs4244285, 19154G>A) significantly influenced primaquine metabolism. CYP1A2 (rs762551, -163C>A) did not influence primaquine metabolism. In haplotypic analysis, significant differences in K_e ($p = 0.00$) and Cm/Cp ($p = 0.05$) were observed between individuals with polymorphisms, GG-MAO-A (891G>T), CT-CYP2D6 (100C>T) and GG-CYP2C19 (19154G>A), and individuals with polymorphisms, TT-MAO-A (891G>T), TT-CYP2D6 (100C>T) and AA-CYP2C19 (19154G>A), as well as polymorphisms, GG-MAO-A (891G>T), TT-CYP2D6 (100C>T) and GA-CYP2C19 (19154G>A). Thus, individuals with CYP2D6 polymorphisms had slower primaquine metabolism activity. The potential significance of genetic roles in primaquine metabolism and exploration of these might help to further optimise the management of *P. vivax* infection.

Keywords *Plasmodium vivax* · Malaria · Polymorphism · Primaquine

Introduction

An estimated 3.4 billion people, globally, are at risk of malaria, which is caused by the *Plasmodium* parasite (WHO 2015). In Malaysia, there has been a reduction of malaria cases over the years and the incidence has declined from 16.1 per 100,000 population in 2012 to 12.9 per 100,000 population in 2013, with the most common infection due to *Plasmodium vivax* parasite infection (Murray et al. 2013; Ariffin et al. 2016). However, there are unreported incidences among indigenous populations where infection may occur at a higher rate (Noryahati et al. 2001). To that end, despite the large reduction in malaria cases, complete eradication in Malaysia remains challenging. *Plasmodium vivax* has been noted to be a difficult disease to control, largely due to the highly adaptable nature of the parasites involved (Chan et al. 2015; WHO 2015). Among the steps adopted in Malaysia for reducing *P. vivax* are the use of bed nets, patient education, close monitoring of drug resistance and frequent monitoring of parasite elimination. However, despite these strategies, other factors may reduce the efficacy of drugs. Most recently, host genetic differences and the effect on drug mechanisms during antimalarial treatment have gained attention (Roederer et al. 2011; Islahudin et al. 2014).

Section Editor: Ramaswamy Kalyanasundaram

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Treatment of *P. vivax* malaria is dependent on a handful of drugs, mainly the newer artemisinins and the much older quinolines. Despite the effectiveness of artemisinins, combination treatment with quinolines is highly recommended in order to reduce resistance and increase effectiveness (Nimir et al. 2006). The more widely used quinolines are chloroquine and primaquine. Primaquine is used for eliminating hypnozoites in *P. vivax*-infected patients and, up until recently, was the only approved drug for eliminating hypnozoites. The newer, 8-aminoquinoline antimalarial, tafenoquine, has only recently been approved for use in *P. vivax* but has yet to be introduced in most countries (Frampton 2018). In view of this, primaquine remains one of the most used antimalarials in Malaysia (Ariffin et al. 2016). However, recent work has shown that the potential influence of host genetic factors may affect treatment outcomes with primaquine (Roederer et al. 2011).

Primaquine is mainly metabolised in the liver, involving human hepatic monoamine oxide (MAO) and cytochrome P450 (CYP450) enzymes. Although in use for several decades, primaquine's mechanisms of efficacy and toxicity are not well understood and its metabolic profile has not been fully elucidated. Elucidating the fundamentals of primaquine metabolism will help to determine if the drug's efficacy is inextricably linked to the polymorphism of MAO and CYP450, which may improve its clinical efficacy. There is growing evidence of the potential contribution of polymorphism of antimalaria drug metabolising, nuclear receptor and drug transport genes on patients (Bennett et al. 2013; Pett et al. 2014). The implication of MAO-A, CYP2D6, CYP1A2 and possibly other CYP450 polymorphisms in primaquine activation in vivo has been suggested in recent work (Bennett et al. 2013; Marcsisin et al. 2016). This is especially of interest, as differences in genetic polymorphisms of MAO-A, CYP2D6, CYP2C19 and CYP1A2 are common among Asians and have been known to affect drug efficacy in the clinical setting (Teh and Bertilsson 2012; Radhakrishnan et al. 2013; Lerena et al. 2014). The MAO-A enzyme plays a major role in the primary metabolism of primaquine; however, inhibition of the enzyme reduces metabolic efficacy, which suggests possible differences in the activity of MAO-A polymorphism (Jin et al. 2014). This is further strengthened by studies which demonstrate that CYP2D6*10 (100C>T) polymorphisms cause *P. vivax* recurrence due to reduced activity of phenotypes (Bennett et al., 2013; Bright et al., 2013; Brasil et al. 2018). Other enzymes, such as CYP2D6 and CYP1A2, have also been implicated in the metabolism of primaquine, with reduced activity observed in different phenotypes in vitro (Bennett et al. 2013; Jin et al. 2014). This suggests that MAO-A (891G>T), CYP2D6*10 (100C>T), CYP2C19*2 (19154G>A) and CYP1A2*1F (-163C>A) polymorphisms in vivo may reduce the activity of enzymes and, possibly, the metabolism of primaquine, leading to clinical inefficacy.

Most studies on malaria focus on polymorphism in the malaria parasite genes, which leads to combination therapies and genomic-based studies directed toward discovering novel targets and agents. However, the potential contribution of host genetic factors on the fundamental basis of primaquine metabolic mechanisms, particularly those associated with antimalarial metabolism, remains largely unexplored (Howes et al. 2012) and is the basis of this current primaquine study of the local population. Although the effect of CYP2D6 in patients has been demonstrated on a small scale, polymorphisms in CYP2C19, CYP1A2 and MAO-A, which have been found abundant in the Asian population, have not been widely explored in patients. Therefore, this work aims to investigate the effect of genetic polymorphisms involving MAO-A and CYP450 on primaquine metabolism in the local Asian population.

Methods

Study design

This was an interventional study performed on 53 healthy volunteers. Adults aged 18 years and above who had never taken primaquine for the past 2 weeks and were healthy during the study were included, with their informed consent. Laboratory tests on liver, kidney, blood cell counts and glucose-6-phosphate dehydrogenase (G6PD) status were performed before the study was conducted to identify each individual's health level. Pregnant women and those individuals with abnormal laboratory readings were excluded from the study. The number of study samples was based on the frequency of allele genes to be studied, which was between 30 and 60% of the local population (GeneCards® The Human Gene Compendium, Abnova version 3). Using the formula previously described (Hong and Park 2012; Neumann et al. 2014), the number of samples required was determined to be 53.

Ethical approval

Ethics approval was obtained from the Research Ethics Committee (Human), under the Medical and Innovation Research Secretariat, Universiti Kebangsaan Malaysia Medical Center (UKM PPI/111/8/JEP-2016-306). The ethics approval fulfils a study procedure involving healthy individuals and drug use in research interventions under the Helsinki Declaration (World Medical Association 2013). The genetic information to be collected and used during the study period was agreed upon by the study participants. This confidential information was collected and stored privately, according to protocol.

Data collection

Study participants were admitted to the study ward for a period of 12 h. During admission, 10 mL of blood was collected at 0 h for genetic analysis. A single dose of primaquine, 15 mg, was given orally to the study participants, and 10 mL of blood was collected at 8 and 12 h post primaquine administration (Tekwani et al. 2015). For genetic analysis, all blood samples were stored at − 80 °C until the extraction process. For drug analysis, blood samples were centrifuged at 1000× *g* for 10 min (Kim et al. 2004). The plasma was then collected and stored at − 80 °C for liquid chromatography-mass spectrometry (LC-MS) analysis (Kim et al. 2004; Jin et al. 2014).

Genetic analysis

For genetic analysis, blood samples were extracted using the Qiagen DNeasy® Blood & Tissue extraction kit, then stored at − 80 °C. A polymerase chain reaction (PCR) optimisation test was carried out to obtain the optimum temperature for the annealing process of each gene studied (Makmor Bakry 2007). The PCR product was then isolated by gel electrophoresis to obtain DNA fragmentation (Li et al. 2015). The purified PCR product was then analysed through the DNA Sanger sequencing method, using the BigDye® Terminator v3.1 cycle sequencing kit (Applied Biosystems, USA) (Boutin et al. 2000). Materials used for the Sanger sequencing method were Terminator Ready Reaction Mix containing 4 μL 2.5× Ready Reaction Premix, 2 μL 5× BigDye Sequencing Buffer, 10 purified PCR products, 3.2 pmol primary and deionised water to achieve the reaction mixture solution of 20 μL. The reaction mixture solution was then incorporated into a 96-capillary sequencer 3730xl DNA Analyser (Applied Biosystems, USA, and Thermo Fisher Scientific producer). The separation of DNA fragments was produced through capillary electrophoresis. The Sequence Scanner v2.0 software then translated the results of the 96-capillary 3730xl DNA Analyser into peaks that represented each nucleotide, A-adenine (green), G-guanine (black), T-thymine (red) and C-cytosine (blue), as well as sequential data, in the form of file formats that can potentially be compared with reference sequences to confirm the polymorphism present (Applied Biosystems 2002).

Drug analysis

The extraction of drug from plasma samples was performed and resulted in a recovery percentage exceeding 80%, according to the guidelines of the European Medicines Agency (2011). A total of 100 μL plasma was added to 300 μL acetonitrile containing 500 ng of ketoconazole (internal standard), vortexed for 30 s and centrifuged at 14,000 rpm for 15 min. A total of 200 μL of the supernatant was dried for 3 h at 30 °C (Eppendorf Concentrator 5301). The crystals were then dissolved in 50 μL of mobile phase A (0.1% formic acid) and B (methanol) at a ratio of 50:50. Three microlitres of this solution was injected into the column (Eclipse Plus C18 Rapid Resolution HD column of 2.1 mm × 50 mm, 1.8 μm Agilent), as previously described (Kim et al. 2004). The mass spectrometry optimisation for each of the analytes was performed using Agilent MassHunter Quantitative B.06.00. The sample injection passage rate was set to 0.25 mL/min. The decomposition rates of the mobile phases were noted. The decomposition of mobile phase A was 0–35% for 4.75 min then 70% until 4.90 min, and, in the same period, the decomposition of mobile phase B was 65 to 30%. Chromatographic conditions were balanced for 2 min, and the peak detection times for primaquine, ketoconazole and carboxyprimaquine were 2.7, 3.3 and 4.1 min, respectively.

Data analyses

Statistical analyses were performed using the IBM Statistical Package for the Social Sciences (SPSS) (version 23) (IBM Corp., Armonk, NY, USA). A *t* test and ANOVA was performed to analyse difference in means between two groups, and more than two groups, respectively. The constant elimination rate (K_e) of primaquine was calculated, as previously described (Kim et al. 2004; Tekwani et al. 2015). The half-life of primaquine elimination used was 3.76 ± 1.8 per hour whilst carboxyprimaquine was 15.7 ± 12.2 per hour (Kim et al. 2004). In this study, the ratio of Cm/Cp refers to the ratio of the level of carboxyprimaquine metabolite to the primaquine parent at 12 h after administration of a single dose of primaquine (Chitnis et al. 2013). A p value of < 0.05 was considered significant.

Results

Demographics

A total of 53 participants were involved in the study. The mean age was 31.2 ± 10.0 years (22 to 58 years old). The majority of participants were Malay ($n = 48$, 90.6%), followed by Chinese ($n = 5$, 9.4%). The numbers of male and female participants were similar (male, $n = 27$; 51% and females, $n = 26$; 49%).

Primaquine metabolism at MAO-A (891G>T)

Primaquine K_e was not affected by MAO-A allele groups ($p = 0.14$; 95% confidence interval − 0.01; 0.05) or MAO-A genotypes ($p = 0.43$) (Table 1). However, there were significant differences in Cm/Cp ratios between the alleles, G and T ($p = 0.01$; 95% confidence interval 0.753; 5.16). There were also significant differences in Cm/Cp ratios between the genotype groups: GG, GT and TT ($p = 0.05$). A Bonferroni post hoc *t* test analysis demonstrated that there was a difference between the GG and TT homozygous genotype variants ($p = 0.05$; 95% confidence

Table 1 Analysis of primaquine and carboxyprimaquine at MAO-A (891G>T) ($N = 53$)

MAO-A (891G>T)	Primaquine (μg/μL) (mean ± SD)		Carboxyprimaquine (μg/μL) (mean ± SD)	
	8 h	12 h	8 h	12 h
Allele				
G	53.89 ± 11.40	34.03 ± 9.99	571.30 ± 114.35	634.72 ± 138.00
T	57.80 ± 15.12	40.04 ± 11.70	574.15 ± 122.02	618.28 ± 126.60
Genotype				
GG	52.64 ± 8.08	33.27 ± 9.28	570.57 ± 109.75	628.57 ± 137.42
GT	57.73 ± 18.09	37.62 ± 12.51	573.52 ± 133.88	655.87 ± 144.91
TT	57.85 ± 12.90	41.02 ± 11.31	574.69 ± 117.66	584.34 ± 104.84
	Frequency		K_e (mean ± SD)	Cm/Cp (mean ± SD)
Allele				
G	0.65		0.12 ± 0.07	19.65 ± 5.66
T	0.35		0.10 ± 0.05	16.69 ± 5.05
p value	–		0.14	0.01
Genotype				
GG	26 (50%)		0.12 ± 0.07	19.96 ± 5.74
GT	17 (32%)		0.11 ± 0.6	18.68 ± 5.61
TT	10 (18%)		0.09 ± 0.05	15.00 ± 4.00
p value	–		0.43	0.05

$p < 0.05$ is considered significant

interval − 0.04; − 1.27). Individuals with a TT genotype variant had a lower Cm/Cp and were shown to be weaker primaquine metabolisers compared to those with the GG genotype. A low Cm/Cp was depicted as having a high primaquine and low carboxyprimaquine level.

Primaquine metabolism at CYP2D6*10 (100C>T)

There was a significant difference observed in the K_e of CYP2D6 allele groups, C and T ($p = 0.03$; 95% confidence interval 0.00; 0.05), and genotype groups, CC, CT and TT ($p = 0.01$) (Table 2). In applying the Bonferroni t test, there was a significant difference between the TT and CT heterozygous genotype variants ($p = 0.01$; 95% confidence interval 0.01; 0.12). There was also a significant difference in Cm/Cp ($p = 0.00$; 95% confidence interval 1.41; 5.57). The difference in Cm/Cp was observed between the TT and CC homozygous genotype variants ($p = 0.06$, 95% confidence interval 1.37; 10.12) as well as the TT and CT heterozygous genotype variants ($p = 0.007$; 95% confidence interval 1.34; 10.37). This demonstrates that individuals carrying the alleles T and the TT genotype variant had reduced enzyme activity; thus, less primaquine was biotransformed into an active metabolite.

Primaquine metabolism at CYP1A2*1F (-163C>A)

There were no significant differences between the K_e of CYP1A2 allele groups, C and A ($p = 0.61$; 95% confidence interval − 0.012, 0.038), and CYP1A2 genotype groups, CC, CA and AA ($p = 0.59$) (Table 3). There were also no significant difference between the Cm/Cp ratio of allele groups, C and A ($p = 0.451$; 95% confidence interval − 0.836; 3.49), and CYP1A2 genotype groups, CC, CA and AA ($p = 0.56$).

Primaquine metabolism at CYP2C19*2 (19154G>A)

There was a significant difference in the K_e of CYP2C19 between alleles, G and A ($p = 0.00$; 95% confidence interval 0.02; 0.07) (Table 4). Genotype analysis showed that there was a significant difference between CYP2C19 genotype groups, namely GG, GA and AA ($p = 0.00$). A Bonferroni t test analysis demonstrated that the significant difference was between the GG and GA heterozygous genotype variants ($p = 0.00$; 95% confidence interval 0.015; 0.10) and GG with the AA homozygous genotype variant ($p = 0.02$; 95% confidence interval 0.010; 0.12). The Cm/Cp was not affected by the allele group ($p = 0.06$; 95% confidence interval − 0.149; 4.29) or genotype group ($p = 0.19$).

Primaquine metabolism and CYP2D6*10, CYP2C19*2 and MAO-A (891G>T) combination

The combined polymorphism of the genes studied and the effects on primaquine metabolism were analysed (Table 5). There was a significant difference in the K_e ($p = 0.00$) and Cm/Cp ratio ($p = 0.05$) of the combined genetic polymorphism. Combined genetic polymorphisms were coded (Table 5). Significant differences in K_e and Cm/Cp were

Table 2 Analysis of primaquine and carboxyprimaquine at CYP2D6*10 (100C>T) ($N = 53$)

CYP2D6*10	Primaquine (μg/μL) (mean ± SD)		Carboxyprimaquine (μg/μL) (mean ± SD)	
	8 h	12 h	8 h	12 h
Allele				
C	54.02 ± 11.01	33.22 ± 11.01	54.02 ± 11.01	33.22 ± 11.01
T	56.87 ± 14.95	39.91 ± 12.67	56.87 ± 14.95	39.91 ± 12.67
Genotype				
CC	53.38 ± 9.84	33.40 ± 7.02	53.38 ± 9.84	33.40 ± 7.02
CT	55.52 ± 13.78	33.36 ± 12.19	55.52 ± 13.78	33.36 ± 12.19
TT	57.73 ± 16.14	43.77 ± 11.32	57.73 ± 16.14	43.77 ± 11.32
		Frequency	K_e (mean ± SD)	Cm/Cp (mean ± SD)
Allele				
C		0.56	0.12 ± 0.06	20.13 ± 5.45
T		0.43	0.09 ± 0.07	16.64 ± 5.24
p value		–	0.03	0.00
Genotype				
CC		21 (39%)	0.12 ± 0.05	20.10 ± 5.27
CT		18 (34%)	0.14 ± 0.08	20.20 ± 6.15
TT		14 (26%)	0.07 ± 0.05	14.35 ± 2.88
p value		–	0.01	0.00

$p < 0.05$ is considered significant

observed between individuals with a combination of BAA-coded polymorphisms, i.e., genotype CT-CYP2D6 (100C>T), GG-CYP2C19 (19154G>A) and GG-MAO-A (891G>T), and individuals with a combination of CCC-coded polymorphisms, i.e., genotype TT-CYP2D6 (100C>T), AA-CYP2C19 (19154G>A) and TT-MAO-A (891G>T), as well as CBA polymorphisms, i.e., genotype TT-CYP2D6 (100C>T), GA-CYP2C19 (19154G>A) and

Table 3 Analysis of primaquine and carboxyprimaquine at CYP1A2*1F (-163C>A) ($N = 53$)

CYP1A2*1F	Primaquine (μg/μL) (mean ± SD)		Carboxyprimaquine (μg/μL) (mean ± SD)	
	8 h	12 h	8 h	12 h
Allele				
C	54.68 ± 7.62	34.58 ± 7.59	566.54 ± 105.56	642.45 ± 137.72
A	55.77 ± 16.26	37.51 ± 13.17	577.43 ± 126.22	616.95 ± 130.16
Genotype				
CC	55.55 ± 7.44	33.82 ± 6.12	548.21 ± 85.54	649.91 ± 140.90
CA	53.38 ± 8.08	35.72 ± 9.55	594.02 ± 128.94	631.27 ± 139.42
AA	57.10 ± 19.65	38.50 ± 15.05	568.22 ± 127.39	609.00 ± 127.89
		Frequency	K_e (mean ± SD)	Cm/Cp (mean ± SD)
Allele				
C		0.47	0.12 ± 0.06	19.32 ± 5.34
A		0.53	0.10 ± 0.07	17.99 ± 5.82
p value		–	0.61	0.45
Genotype				
CC		15 (28%)	0.12 ± 0.56	19.67 ± 4.88
CA		20 (38%)	0.11 ± 0.07	18.77 ± 6.14
AA		18 (34)	0.10 ± 0.07	17.56 ± 5.76
p value		–	0.59	0.56

$p < 0.05$ is considered significant

Table 4 Analysis of primaquine and carboxyprimaquine at CYP2C19*2 (19154G>A) ($N = 53$)

CYP2C19*2	Primaquine (μg/μL) (mean ± SD)		Carboxyprimaquine (μg/μL) (mean ± SD)	
	8 h	12 h	8 h	12 h
Allele				
G	54.68 ± 7.62	34.58 ± 7.59	566.54 ± 105.56	642.45 ± 137.72
A	55.77 ± 16.26	37.51 ± 13.17	577.43 ± 126.22	616.95 ± 130.16
Genotype				
GG	57.90 ± 12.71	33.48 ± 11.79	589.14 ± 118.58	633.62 ± 133.85
GA	53.80 ± 14.22	38.16 ± 9.21	546.51 ± 105.11	630.83 ± 133.69
AA	51.68 ± 10.67	38.61 ± 11.83	580.86 ± 137.52	614.35 ± 150.08
		Frequency	K_e (mean ± SD)	Cm/Cp (mean ± SD)
Allele				
G		0.63	0.13 ± 0.07	19.38 ± 5.37
A		0.37	0.08 ± 0.5	17.31 ± 5.85
p value		–	0.00	0.06
Genotype				
GG		24 (45%)	0.14 ± 0.07	20.18 ± 5.23
GA		19 (36%)	0.09 ± 0.05	17.34 ± 5.42
AA		10 (19%)	0.08 ± 0.60	17.27 ± 6.54
p value		–	0.00	0.19

$p < 0.05$ is considered significant

genotype GG-MAO-A (891G>T). Thus, individuals with CCC and CBA polymorphisms had a primaquine metabolism activity that was slower than BAA individuals.

Discussion

The pharmacogenetic approach in malaria management has evolved since the discovery of the role of G6PD during primaquine administration (Howes et al. 2012). Since then, various works have been undertaken to determine other genetic factors that may predetermine the efficiency of pharmacological management. This is especially relevant for liver hypnozoite eradication with primaquine, due to the limited pharmacological options available during *P. vivax* treatment. Although it is understood that MAO-A acts as the primary enzyme of primaquine metabolism into carboxyprimaquine (Constantino et al. 1999; Marcsisin et al. 2016), work has shown that CYP450 enzymes are also involved in the production of its intermediate metabolites (Constantino et al. 1999).

Table 5 Analysis of primaquine metabolism in CYP2D6*10 (100C>T), CYP2C19*2 (19154G>A) and MAO-A (891G>T) combination ($N = 53$)

CYP2D6	CYP2C19	MAO-A	Polymorphism code	*n* (%)	K_e (mean ± SD)	Cm/Cp (mean ± SD)
CC	GG	GG	AAA	7 (13.2)	0.12 ± 0.04	18.8 ± 4.7
CC	GG	GT	AAB	4 (7.5)	0.15 ± 0.07	21.4 ± 6.9
CC	GA	GG	ABA	4 (7.5)	0.12 ± 0.07	21.5 ± 5.8
CC	AA	GT	ACB	3 (5.7)	0.08 ± 0.06	19.8 ± 5.6
CT	GG	GG	BAA	5 (9.4)	0.22 ± 0.06	25.1 ± 2.2
CT	GG	GT	BAB	3 (5.7)	0.16 ± 0.10	18.4 ± 6.6
CT	GA	GG	BBA	3 (5.7)	0.07 ± 0.04	18.1 ± 8.2
CT	GA	GT	BBB	4 (7.5)	0.07 ± 0.04	14.6 ± 2.5
TT	GA	GG	CBA	3 (5.7)	0.05 ± 0.02	13.8 ± 2.6
TT	AA	TT	CCC	5 (9.4)	0.06 ± 0.06	13.1 ± 4.2

The polymorphism code for CYP2D6*10 (100C>T), CYP2C19*2 (19154G>A) and MAO-A (891G>T) ranges from AAA (wild type of all three genes) to CCC (homozygous variant of all three genes). A represents wild-type CC CYP2D6*10 (100C>T)/GG CYP2C19*2 (19154G>A)/GG MAO-A (891G>T). B represents heterozygous variant CT CYP2D6*10 (100C>T)/GA CYP2C19*2 (19154G>A)/GT MAO-A (891G>T). C represents homozygous variant TT CYP2D6*10 (100C>T)/AA CYP2C19*2 (19154G>A)/TT MAO-A (891G>T)

As MAO-A is found in most tissues compared to CYP450, the MAO-A effects on primaquine metabolism are observed much more clearly. However, both MAO-A and CYP450 contribute equally to primaquine metabolism (Constantino et al. 1999). If we take into account the effects of genetic polymorphisms of both MAO-A and CYP450 enzymes present in an individual, it is possible that the metabolism may, potentially, vary from one individual to another (Bennett et al. 2013; Bright et al. 2013), as clearly demonstrated in this current work. This is also supported by previous data that demonstrate that the individual responses to drugs are very much dependent on their genetic state (Cho and Yoon 2018). To the best of our knowledge, this is the first time work has been conducted to show the differences in primaquine metabolism and its effects in MAO-A, CYP2D6, CYP1A2 and CYP2C19 polymorphism among healthy volunteers in the local population.

The MAO enzyme plays an important role in catalysing various biological pathways, including drugs such as primaquine (Berlin and Anthenelli 2001). In in vitro studies, MAO-A inhibitors lower the levels of the primaquine metabolite, carboxyprimaquine, suggesting an active role in primaquine metabolism (Jin et al. 2014). We were able to demonstrate a similar occurrence among healthy volunteers with MAO-A polymorphisms, which further strengthens the discovery of MAO-A's influence on drug metabolism, as previously studied (Constantino et al. 1999; Pybus et al. 2012). Individuals with a homozygous genotype variant had a slower primaquine metabolism compared to wild type.

Among the CYP450 genes studied were those that were commonly observed within the population (Ang et al. 2016; Yu et al. 2017). The present work demonstrated that both CYP2D6 had an effect on primaquine metabolism in healthy volunteers, and this suggests that it may most likely occur in patients treated for *P. vivax* malaria. Differences in metabolism are further supported in work that demonstrates treatment failure occurring in patients with CYP2D6 polymorphisms, due to the slower primaquine metabolism occurring in the variant genotype (Bennett et al., 2013; Bright et al. 2013). The current in vivo data also suggests an effect in CYP2C19 polymorphism. It is quite possible, however, that the effect of CYP2C19 on primaquine metabolism is not related to the formation of carboxyprimaquine but, potentially, a different active metabolite (Pybus et al. 2012). Therefore, further studies are recommended to understand the therapeutic result of CYP2C19 polymorphism on primaquine. The significance of these findings regarding CYP450 rests in the high prevalence of CYP2D6 and CYP2C19 polymorphisms in the Asian region (Sen 2016), as well as indigenous people in the country (Ang et al. 2016; Yu et al. 2017).

Following this, however, it is vital that the overall clinical effect of different polymorphisms is ascertained (Chitnis et al. 2013) to determine the effects of pharmacogenetics during primaquine treatment. The present work demonstrated that the combination of homozygous MAO-A, CYP2D6 and CYP2C19 variant genotypes was found to be less active in forming carboxyprimaquine metabolites. It is also interesting to note that the presence of a CYP2D6 homozygous variant genotype significantly reduces primaquine metabolism, suggesting a dominant role of CYP2D6 in primaquine metabolism during treatment compared to other genes studied. To that end, the role of MAO-A and both CYP2D6 and CYP2C19 polymorphisms as factors in possibly determining *P. vivax* therapeutic outcomes should be taken into consideration, in order to ensure appropriate management (Marcsisin et al. 2016).

Overall, this study demonstrated the influence of MAO-A, CYP2D6 and CYP2C19 genetic polymorphisms on primaquine metabolism, albeit with a few limitations. Firstly, the work was done in a small group of healthy individuals, which could be further strengthened by performing nationwide data collection. In addition, in order to understand the kinetic activity of primaquine, measurements were performed on only primaquine and carboxyprimaquine and, hence, a more detailed study, particularly involving parameters of formation of other metabolites, could be performed in the future (Cho and Yoon 2018). Despite this, the aim of this study was successfully achieved, and it was demonstrated that the differences in primaquine metabolism may contribute to the incidence of *P. vivax* recurrence, among other factors, especially involving local citizens (Ariffin et al. 2017). The impact of this on existing comprehensive efforts is to possibly develop a genetic screening process in an attempt to improve the management of *P. vivax* and reduce therapeutic failure and possible recurrence.

Funding This study was funded by the Ministry of Higher Education, Malaysia, under the Fundamental Research Grant Scheme [FRGS/1/2016/SKK09/UKM/02/1].

Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author upon reasonable request.

Compliance with ethical standards

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

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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