Drug Metabolism and Pharmacokinetics 57 (2024) 101029

Contents lists available at ScienceDirect

Drug Metabolism and Pharmacokinetics

journal homepage: www.journals.elsevier.com/drug-metabolism-and-pharmacokinetics

Regular Article

Genetic variation present in the *CYP3A4* gene in Ni-Vanuatu and Kenyan populations in malaria endemicity

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ARTICLE INFO

Keywords:
Cytochrome P450
CYP3A4
Genetic variation
Pharmacogenetic
SNPs
Antimalarial
Kenya
Vanuatu

ABSTRACT

Cytochrome P450 3A4 (CYP3A4) enzyme is involved in the metabolism of about 30 % of clinically used drugs, including the antimalarials artemether and lumefantrine. *CYP3A4* polymorphisms yield enzymatic variants that contribute to inter-individual variation in drug metabolism. Here, we examined *CYP3A4* polymorphisms in populations from malaria-endemic islands in Lake Victoria, Kenya, and Vanuatu, to expand on the limited data sets. We used archived dried blood spots collected from 142 Kenyan and 263 ni-Vanuatu adults during cross-sectional malaria surveys in 2013 and 2005–13, respectively, to detect *CYP3A4* variation by polymerase chain reaction (PCR) and sequencing. In Kenya, we identified 14 *CYP3A4* single nucleotide polymorphisms (SNPs), including the 4713G (*CYP3A4*1B*; allele frequency 83.9 %) and 19382A (*CYP3A4*15*; 0.7 %) variants that were previously linked to altered metabolism of antimalarials. In Vanuatu, we detected 15 SNPs, including the 4713A (*CYP3A4*1A*; 88.6 %) and 25183C (*CYP3A4*18*; 0.6 %) variants. Additionally, we detected a rare and novel SNP C4614T (0.8 %) in the 5′ untranslated region. A higher proportion of *CYP3A4* genetic variance was found among ni-Vanuatu populations (16 %) than among Lake Victoria Kenyan populations (8 %). Our work augments the scarce data sets and contributes to improved precision medicine approaches, particularly to anti-malarial chemotherapy, in East African and Pacific Islander populations.

1. Introduction

Cytochromes P450 (CYPs) comprise a superfamily of enzymes crucial to the metabolism of drugs and xenobiotics in mammals [1,2]. In humans, CYP3A4 is abundantly expressed in the liver and the intestines and is involved in the metabolism of about 30 % of clinically prescribed drugs [3–5]. CYP3A4 has been shown to have an impact on the pharmacokinetics, toxicities and clinical outcomes of several drugs, including antimalarial drugs (lumefantrine, artemether [6], halofantrine [7–9], quinine [10]), immunosuppressive agents (cyclosporine and tacrolimus [11]), cardiovascular drugs (ticagrelor and prasugrel [12]), painkillers [13], and antivirals [14]. Additionally, CYP3A4 is involved in the elimination of drugs, xenobiotics, and other endogenous molecules from the body [2]. Together with the isoform CYP3A5, it is involved in the metabolism of over 50 % of known CYP450 substrates [15].

CYP3A4 activity levels show great inter-individual variations due to both genetic and non-genetic factors including food-drug and drug-drug interactions. Genetic variations in both coding and core regulatory sites of the *CYP3A4* gene are thought to be the key drivers of inter-individual

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https://doi.org/10.1016/j.dmpk.2024.101029
Received 11 April 2024; Received in revised form 1 July 2024; Accepted 8 July 2024
Available online 10 July 2024

variation since they affect its expression or function [2]. Missense mutations in the coding regions are rare but have been shown to alter enzyme kinetics using various substrates *in vitro* [10,16–18]. Other rare missense mutations and insertions in the coding regions lead to premature stop codons [19–22]. Polymorphisms in non-coding regions have been shown to affect *CYP3A4* expression [23,24]. The Pharmacogene Variation Consortium includes 41 missense variants in the coding regions and two non-coding variants known to affect *CYP3A4* expression.

Compared to other continental regions, there is a high level of inter-individual and inter-ethnic *CYP* genetic diversity in Africa [25,26], which can result in differences in clinical outcomes [27]. The frequencies of the *CYP3A4* 4713G variant, which defines the *CYP3A4*1B* allele, are notably higher in African populations [15,28,29]. This allele has been shown to enhance drug elimination [30] and affect the metabolism of antimalarial drugs [6]. It is also associated with reduced metabolism of anti-cancer drugs among patients [31]. Since CYP3A4 is involved in the metabolism of vitamin D, rickets is suggested to have selected against *CYP3A4*1B* in non-African populations [32].

Malaria is endemic in East Africa and the Western Pacific, yet very few ethnically specific *CYP3A4* polymorphism data are available for East African and Pacific Islander populations despite their potential contribution to the pharmacokinetics and clinical outcome of antimalarial treatment [6–10]. While previous studies have described *CYP3A4* variation in different African populations [33–35], most of the studies have relied on relatively small samples and focused on a few known single nucleotide polymorphisms (SNPs) with clinical importance. On the other hand, to our knowledge, no effort has been made to examine *CYP3A4* variation among Pacific Islanders. As a result, a gap exists in translating pharmacogenetic data into actionable advice, such as applying personalized medicine that could help minimize adverse drug reactions and improve treatment outcomes [36,37].

We previously identified and characterized the functions of several novel *CYP2D6* allelic variants among island populations in Lake Victoria, Kenya, and Vanuatu [38], suggesting that *CYP3A4* variants specific to these populations may exist. Therefore, our goals were to sequence all thirteen *CYP3A4* exons and part of the 5′ untranslated region (UTR) and introns and to characterize the effects of novel non-synonymous variants *in vitro*. By expanding the limited data set available, we sought to improve precision medicine approaches, particularly to anti-malarial chemotherapy in East African and Pacific Islander populations.

2. Methodology

2.1. Ethical considerations

The Ethics Committee of Tokyo Women's Medical University and the Ministry of Health in Vanuatu approved the study. In Kenya, the ethical approval was obtained from the Kenyatta National Hospital/University of Nairobi-Ethics and Research Committee in Kenya (No. P7/1/2012). Ethical approval to analyse the previously collected samples was provided by the Ethics Committee of Osaka Metropolitan University (Permission number 3206). Briefly, the local interpreters aided in informing the study participants of the purposes and procedures to be conducted, and written informed consent was obtained from all participants.

2.2. Study sites and samples

Samples were obtained from adult participants who enrolled in our community-based cross-sectional malariometric surveys in Kenya in 2013 and Vanuatu in 2005–2013. In Kenya, participants were recruited from four islands in Lake Victoria (Mfangano, Kibuogi, Ngodhe, and Takawiri) and one lakeshore community (Ungoye) in Homa Bay County (Fig. 1). Malaria is endemic in all sites, although the transmission intensity differs [39]. The major ethnic groups are Luo and Suba. In Vanuatu, participants were recruited from six islands across the archipelago: Torres Islands, Ambae, Epi, Tanna, Futuna, Tanna, and Aneityum (Fig. 2). Malaria was historically endemic in all islands except Futuna, where no *Anopheles* vectors are present [40]. Ethnically, the ni-Vanuatu populations on all sampled islands are Melanesian except Futuna, which is Polynesian [41,42]. Capillary blood samples (70 μl) were obtained by fingerpick using heparinized microhematocrit capillary tubes (Fisherbrand) and blotted on Whatman 31 ET Chr filter paper (Cytiva). Blood spots were allowed to dry at ambient temperature and dried blood spots (DBS) were individually stored in zipped plastic bags with desiccant.

2.3. CYP3A4 genotyping

2.3.1. DNA extraction

DNA was extracted from dried blood spots using the QIAamp DNA Mini Kit (Qiagen, Japan) per the manufacturer's instructions. For internal quality control measures, we employed both negative and positive controls throughout DNA extraction, PCR amplification, and PCR

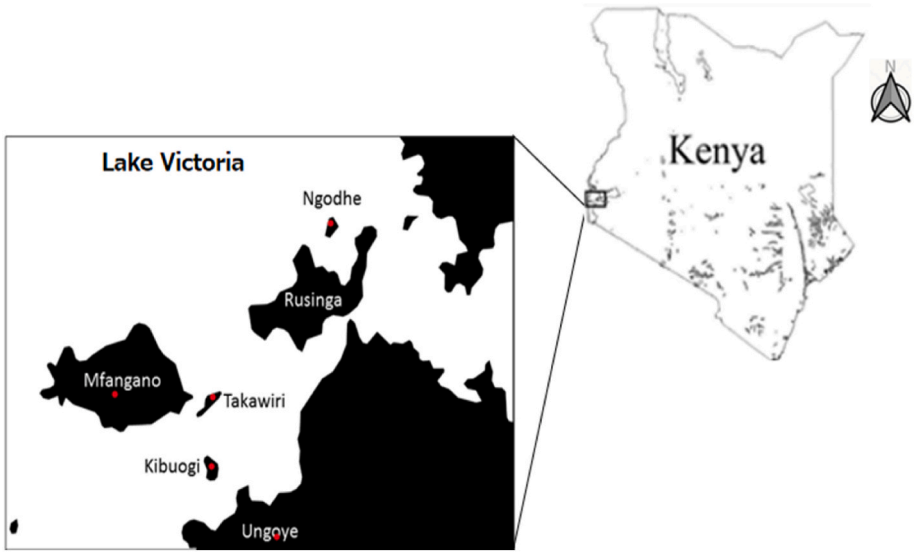

Fig. 1. Map of the five study sites (Kibuogi, Mfangano, Ngodhe, Takawiri, Ungoye) in Lake Victoria, Homabay County, Kenya.

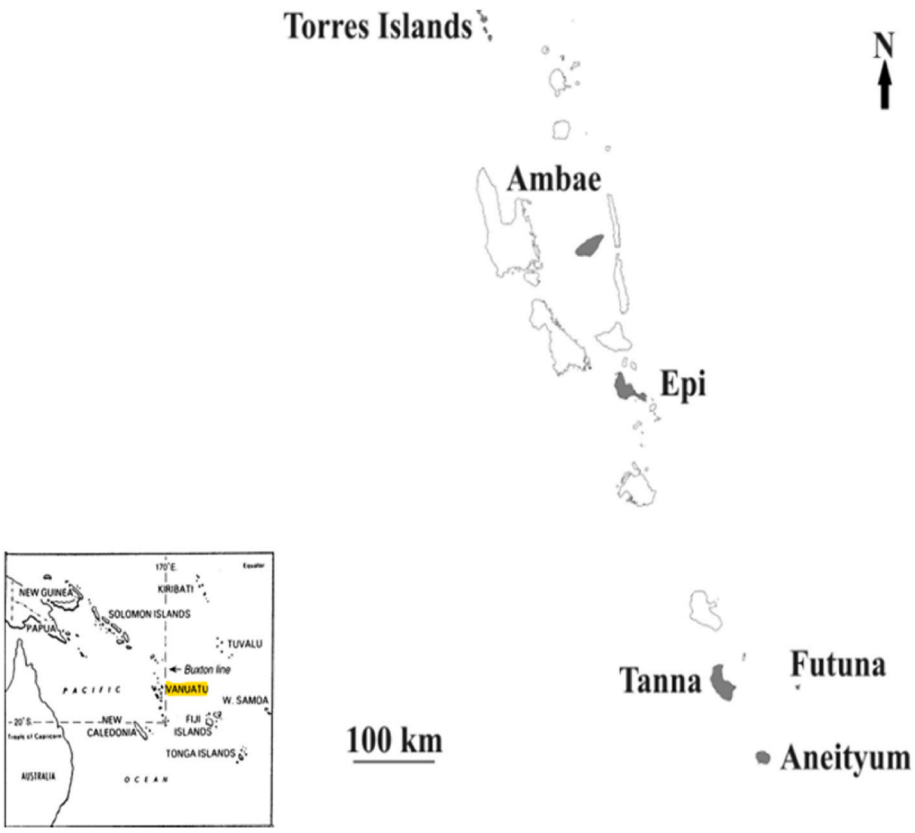

Fig. 2. Map of the six study sites (Ambae, Aneiytum, Epi, Futuna, Tanna and Torres Islands) in Vanuatu.

product purification processes.

2.3.2. PCR amplification and DNA sequencing

We amplified the thirteen *CYP3A4* exons and the 5' UTR using PCR primers previously described by Kumondai et al. [16]. Briefly, PCR amplifications of each exon were carried out in a 20-μl reaction consisting of 10 μl of PrimeSTAR® Max Premix (TAKARA BIO INC), 0.3 μM of respective primers, and 1.0 μl of template DNA. For exons 1 and 13, the optimized cycling conditions consisted of 37 amplification cycles at 98 °C for 10 s, 65.2 °C for 5 s, and 72 °C for 10 s. For the remaining exons, the optimized cycling conditions consisted of 35 amplification cycles at 98 °C for 10 s and 70 °C for 15 s.

The amplified PCR products (5 μl) were then resolved in 1.5 % agarose gel electrophoresis to confirm the amplification's success. We then purified 10 μl of the remaining PCR products using the ExoSAP-IT PCR Product Cleanup Reagent (Thermo Fischer Scientific) per the manufacturer's instructions before bi-directional Sanger sequencing using the BigDye® Terminator v3.1 Cycle Sequencing Kit on the ABI 3730xl analyzer.

2.3.3. Data analysis

The raw chromatograms from forward and reverse sequences for the respective sample were trimmed to remove PCR primers and aligned to the reference *CYP3A4* sequence (GenBank accession NG_008421.1) to identify genetic variants using Geneious Prime® 2023.1.1. The variants were then compared to the NCBI SNP database (dbSNP; https://www.ncbi.nlm.nih.gov/snp/) and the 1000 Genomes Browser (https://www.internationalgenome.org/1000-genomes-browsers/index.html) for the identification of the known and novel variants. *CYP3A4* haplotypes were reconstructed using PHASE v2.1 [43]. Alleles were named according to the Human Cytochrome P450 *CYP3A4* Allele Nomenclature Database (https://www.pharmvar.org/gene/CYP3A4). Principal coordinates analysis (PCoA) and partitioning of *CYP3A4* genetic variance at different hierarchical levels were estimated using analysis of molecular variance (AMOVA) as implemented in GenAlEx 6.5 [44].

3. Results

3.1. CYP3A4 variation in the Lake Victoria Kenyan populations

One hundred forty-two (142) individuals from four islands and one lakeshore community were successfully genotyped for *CYP3A4* (Table 1). We identified 14 *CYP3A4* SNPs: one in the 5' UTR, one in exon 6, and twelve in introns. The 4713G (rs2740574) variant, which defines the *CYP3A4*1B* allele, was found in all populations, lowest in Ungoye at a frequency of 79.7 % and highest in Kibuogi at 89.5 % (Table 2). The frequency of the 4713G variant in the Lake Victoria Kenyan populations was slightly higher than the aggregate frequency for African populations reported by the 1000 Genome Project (Fig. 3). The G19382A (rs4986907) SNP in exon 6, which results in the amino acid substitution of arginine by glutamine at residue 162 (R162Q), was found only on Kibuogi at 2.6 % and Ngodhe at 2.5 % (Table 2).

We inferred a total of forty-nine *CYP3A4* haplotypes corresponding to three alleles (**1A*, **1B*, and **15*) from 14 SNPs (Supplemental Table 1). *CYP3A4*1B* was the most frequently observed allele at 82.4 %, followed by *CYP3A4*1A* and *CYP3A4*15* at 16.9 % and 0.7 %, respectively.

AMOVA revealed that 8 % of *CYP3A4* genetic variance was found among the population and 92 % was found within the population (Table 4). PCoA based on *CYP3A4* genetic distances showed two clusters, with Kibuogi being most dissimilar to the other four populations (Fig. 4). The first and second dimensions explained 92.1 % and 7.9 % of the total variation, respectively.

Table 1
Demographic characteristics of five Lake Victoria Kenyan and six ni-Vanuatu populations.

Kenya (n = 142)						Vanuatu (n = 263)					
Population	Kibuogi (n = 19)	Mfangano (n = 53)	Ngodhe (n = 20)	Takawiri (n = 18)	Ungoye (n = 32)	Ambae (n = 46)	Aneityum (n = 37)	Epi (n = 45)	Futuna (n = 44)	Tanna (n = 44)	Torres Islands (n = 47)
Male (%)	10 (52.6)	26 (49.1)	10 (50.0)	10 (55.6)	16 (50.0)	20 (43.5)	18 (48.6)	23 (51.1)	22 (50.0)	21 (47.7)	22 (46.8)
Median age in years (IQR[a])	33 (6.5)	38 (10)	38 (8.5)	36.5 (15.5)	40 (14.5)	46.5 (13.8)	44 (4)	45 (17)	38 (18)	37 (19.2)	40 (9)

[a] IQR - interquartile range.

Table 2
Frequencies (%) of *CYP3A4* variants in five Lake Victoria Kenyan populations.

Position[a]	SNP ID	Region	Allele	Kibuogi	Ngodhe	Takawiri	Mfangano	Ungoye	Overall (95 CI)
4713 G > A	rs2740574	5′ UTR	*1A	10.53	15.00	13.87	18.87	20.31	16.09 (10.05,22.13)
19382 G > A	rs4986907	Exon 6	*15	2.63	2.50	0.00	0.00	0.00	0.70 (0,2.07)
20741 C > T	rs4987159	Intron 7		13.16	2.50	0.00	0.00	4.69	3.17 (0.29,6.05)
20866 G > T	rs2687116	Intron 7		7.89	10.00	8.33	11.32	9.38	9.86 (4.96,14.76)
20950 T > A	rs12721622	Intron 7		10.53	0.00	5.56	0.00	15.63	5.63 (1.84,9.42)
22137 C > T	rs12721624	Intron 8		0.00	0.00	2.78	0.00	0.00	0.35 (0,1.32)
22180 C > T	rs147729006	Intron 8		0.00	2.50	0.00	0.00	0.00	0.35 (0,1.32)
22996 C > G	rs10267228	Intron 9		10.53	0.00	0.00	3.77	18.75	7.04 (2.83,11.25)
25343 G > A	rs2242480	Intron 10		78.95	55.00	66.67	77.36	89.06	75.70 (68.65,82.75)
25378 C > T	rs4986912	Intron 10		0.00	0.00	0.00	0.00	1.56	0.35 (0,1.32)
25422 G > C	rs4986911	Intron 10		0.00	0.00	5.56	1.89	4.69	1.76 (0,3.92)
25440 T > C	rs34738177	Intron 10		0.00	5.00	2.78	1.89	0.00	2.46 (0,5.01)
28194 C > T	rs12721620	Intron 12		18.42	12.50	19.44	17.92	15.63	16.90 (10.74,23.06)
30834 A > G	rs3735451	Intron 13		0.00	65.00	66.67	75.47	82.81	64.44 (56.57,72.31)

[a] Nucleotide position according to *CYP3A4* reference sequence (GenBank accession NG_008421.1). The 95 % confidence interval is shown in parentheses for the overall frequency of each variant.

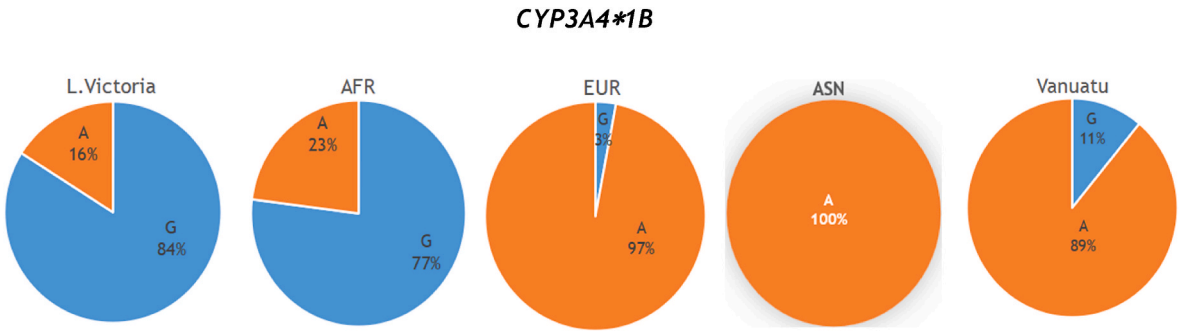

Fig. 3. Distribution of the *CYP3A4* 1B* allele (4713G) frequencies in the Lake Victoria Kenyan, ni-Vanuatu and the three populations present in the 1000 Genomes Browser. (AFR, African; ASN, East Asian; EUR, European).

Fig. 4. - Principal coordinates analysis (PCoA) of pairwise *CYP3A4* genetic distances among five Lake Victoria Kenyan (left) and the six ni-Vanuatu populations (right).

3.2. *CYP3A4 variation in the ni-Vanuatu populations*

Two hundred sixty-three (263) individuals from six islands were successfully genotyped for *CYP3A4* (Table 1). We detected 15 *CYP3A4* SNPs: seven in the 5' UTR, one in exon 10, and seven in introns (Table 3). The 4713A variant, which defines *CYP3A4*1A,* was found at high frequencies (73.0–97.8 %) in all populations. The 4713G variant is absent in Asian populations and rare in European populations (Fig. 3).

Table 3
Frequencies (%) of *CYP3A4* variants in six ni-Vanuatu populations.

Position[a]	SNP ID	Region	Allele	Ambae	Aneityum	Epi	Futuna	Tanna	Torres Islands	Overall (95 % CI)
4576 T > C	rs4986914	5′ UTR		4.35	12.62	0.00	9.09	1.14	0.00	4.18 (1.76,6.6)
4591 A > G	rs944346333	5′ UTR		2.17	1.35	1.11	1.14	1.14	1.14	1.14 (0,2.42)
4614 C > T	Novel	5′ UTR		0.00	0.00	4.44	0.00	0.00	0.00	0.76 (0.2, 1.81)
4713 G > A	rs2740574	5′ UTR	*1A	93.48	72.97	97.78	84.09	94.32	86.17	88.59 (84.75,92.43)
4851 G > C	rs1816010825	5′ UTR		2.17	0.00	0.00	0.00	0.00	0.00	0.38 (0,1.12)
4982 T > C	rs1584553015	5′ UTR		0.00	0.00	2.22	0.00	0.00	1.06	0.57 (0,1.41)
4985 A > G	rs12721633	5′ UTR		5.43	12.16	1.11	5.68	2.27	0.00	4.18 (1.76, 6.6)
11262 T > A	rs12721619	Intron 3		10.87	20.27	3.33	12.50	3.41	0.00	7.98 (4.7, 11.26)
19313 T > A	rs68106838	Intron 6		7.61	17.57	3.33	10.23	0.00	5.32	7.03 (3.94, 10.12)
20866 G > T	rs2687116	Intron 7		3.26	70.27	95.56	86.36	92.05	92.55	73.19 (67.84, 78.54)
22996 C > G	rs10267228	Intron 9		0.00	4.05	4.44	0.00	0.00	0.00	1.33 (0, 2.71)
25183 T > C	rs28371759	Exon 10	*18	0.00	0.00	0.00	0.00	3.41	0.00	0.57 (0,1.41)
25343 C > G	rs2242480	Intron 10		71.74	74.32	22.22	40.91	26.14	36.17	44.49 (38.49, 50.49)
27259 T > C	rs975452961	Intron 11		5.43	12.16	4.44	6.82	1.14	0.00	4.75 (2.18, 7.32)
30834 A > G	rs3735451	Intron 13		38.04	63.51	22.22	50.00	21.59	40.43	38.59 (32.71, 44.47)

[a] Nucleotide position according to *CYP3A4* reference sequence (GenBank accession NG_008421.1). The 95 % confidence interval is shown in parentheses for the overall frequency of each variant.

Other frequently observed SNPs included G20866T (rs2687116; 73.2 %) in intron 7, C25343G (rs2242480; 44.5 %) in intron 10, and A30834G (rs3735451; 38.6 %) in intron 13, although they showed substantial differences in frequencies among islands (Table 3). T25183C (rs28371759) in exon 10, which results in the amino acid substitution of leucine by proline at residue 293 (L293P), was found only on Tanna at 3.4 %. We identified a novel SNP, C4614T in the 5′ UTR, on Epi at a frequency of 4.4 % (Table 3).

Sixty-two (62) *CYP3A4* haplotypes corresponding to three alleles (**1A*, **1B*, and **18*) were inferred from 15 SNPs (Supplemental Table 2). *CYP3A4*1A* was the most frequently observed allele at 88.0 %, followed by *CYP3A4*1B* and *CYP3A4*18* at 11.4 % and 0.6 %, respectively.

AMOVA revealed significant ($p < 0.001$) population structure, with 16 % of *CYP3A4* genetic variance found among populations and 84 % within population (Table 4). Three clusters were observed in the two-dimensional plot of *CYP3A4* genetic distances: Ambae in the lower right quadrant, Aneityum in the upper right quadrant, and the other islands on the left side (Fig. 4). The first and second dimensions explained 63.7 % and 29.2 % of the total variation, respectively.

4. Discussion

Genetic polymorphisms in drug-metabolizing *CYP* genes are known to affect enzyme functions and have clinical relevance [4]. CYP3A4 metabolizes a wide range of pharmaceuticals including antimalarial drugs [6–10]. Genetic variation in *CYP3A4* among populations in malaria-endemic regions has the potential to affect antimalarial treatment outcomes and drug efficacy [45]. We previously identified several novel *CYP2D6* polymorphisms with decreased functions among populations in the Lake Victoria region of Kenya and Vanuatu, where malaria is endemic and pharmacogenetic data were scarce [38]. Here, we examined in the same populations genetic variation in *CYP3A4*. Unlike the polymorphic *CYP2D6*, no novel non-synonymous substitutions were identified in *CYP3A4* among 142 Lake Victoria Kenyan and 263 ni-Vanuatu individuals. The vast majority of *CYP3A4* variants identified in this study were located in introns and the 5' UTR. These observations suggested that *CYP3A4* was conserved in both Lake Victoria Kenyan and ni-Vanuatu populations.

The A4713G SNP (rs2740574) is the first and most frequently typed *CYP3A4* polymorphism due to the high frequencies of the G variant (*CYP3A4*1B*) in African populations [46]. The *CYP3A4*1B* frequency among the Lake Victoria Kenyan populations (83.9 %) was comparable to the reported frequency among East African populations: 83 % in the Luhya ethnic group in Kenya (https://www.ensembl.org/index.html), 78 % in Mwanza [29], and 74 % in Morogoro [28], Tanzania. High frequencies of *CYP3A4*1B* have also been reported in other African countries: 87 % in Nigeria [47], 75 % in Mali [35], and 66 % in South Africa [33]. The frequencies of the *CYP3A4*1B* allele are considerably lower in North African populations: 24 % among Moroccans, 11 % in Tunisia [48], and 20 % in Libya [49]. Extensive contribution of Near Eastern ancestry to North African populations likely contributed to the differentiation of North Africa from the rest of the continent [50].

Literature on the phenotypic effects of the 4713G variant is inconclusive. It has been associated with poor metabolism of antimalarials including artemether, lumefantrine [34,45], piperaquine [6,51], mefloquine [52] and quinine [10]. Artemether-lumefantrine (AL) is the most widely used artemisinin-based combination therapy (ACT) to treat *Plasmodium falciparum* malaria in sub-Saharan Africa, and AL treatment failure has been reported in Angola, Burkina Faso, the Democratic Republic of Congo, and Uganda [53]. Artemether is hydrolyzed to its active metabolite dihydroartemisinin (DHA) by CYP3A4 [6], although it remains unclear if poor artemether metabolism due to the 4713G variant plays a role in the emergence of resistance in sub-Saharan Africa. It is worth noting that both piperaquine and mefloquine are used as partner drugs in other formulations of ACTs, as such the functional effects of the *CYP3A4* 4713G variant may have major implications on malaria disease management and control.

In populations from Kibuogi and Ngodhe, we found low frequencies (2.5 %–2.6 %) of the 19382A variant (*CYP3A4*15*), which were comparable to a study in South Africa [[33]]. This missense mutation might have functional consequences for the CYP3A4 protein as arginine is positively charged and hydrophilic. In contrast, glutamine is neutral and

Table 4
Analysis of molecular variance (AMOVA) for Kenyan and ni-Vanuatu populations.

Kenya						Vanuatu				
Source of Variance	Df	SS	MS	Est. Var.	%	Df	SS	MS	Est. Var	%
Among population	4	15.885	3.971	0.104	8	5	63.608	12.722	0.260	16
Within Population	137	162.249	1.184	1.184	92	257	343.575	1.337	1.337	84
Total	141	178.134		1.288	100	262	407.183		1.597	100

Df-degrees of freedom, SS- sum of squares; MS – mean of squares, Est. Var – Estimated variance.

polar, potentially affecting electrostatic interactions critical for substrate binding [54]. Using a baculovirus-insect cell expression system, Zhou et al. [10] showed that *CYP3A4*15* was associated with rapid metabolism and increased intrinsic clearance of quinine, which might indicate lower therapeutic efficacy when standard doses are administered.

Genome-wide analyses of ancient and present-day populations in Vanuatu indicate complex histories of admixture of East and Southeast Asians, Papuans, and Polynesians at different times over the past 3000 years [55–57]. Furthermore, relatively few studies have examined genetic variation in *CYP* genes among Pacific Islanders, making it difficult to contextualize our findings in the ni-Vanuatu populations. The latter point is made evident by our observation that 11% of the alleles detected in the ni-Vanuatu populations were *CYP3A4*1B*, which is absent in Asian populations (Fig. 3).

In Vanuatu, the frequencies of some *CYP3A4* SNPs (e.g., A20866G, G25343A; Table 3) varied widely among islands. We previously observed a similar pattern of substantial variation in *CYP2C19* alleles among ni-Vanuatu populations on different islands [58,59]. AMOVA indicated that among-island genetic variance was higher in Vanuatu than in Kenya (16 % vs. 8 %; Table 4), likely because of greater degrees of geographic isolation among ni-Vanuatu populations (Fig. 2). Nonetheless, similar *CYP3A4* SNP frequencies were observed among neighboring islands of Futuna, Tanna, and Aneityum (Table 3), even though Futuna is a Polynesian outlier with a culturally distinct population [60].

We detected a low frequency (3.4 %) of the 25183C variant (*CYP3A4*18*) allele in the Tanna population (Table 3). This missense mutation (L293P) has been reported among Koreans [61], Japanese [62] and Han Chinese [63]. The *CYP3A4*18* allele is a rapid metabolizer of sex steroids and is associated with low bone mineral density [64]. Zhou et al. [10] showed that *CYP3A4*18* exhibited decreased intrinsic clearance of quinine *in vitro*.

In line with previous studies [33], most of the SNPs detected in the Lake Victoria Kenyan and ni-Vanuatu populations in this study were in the 5' UTR and introns. However, the significance of intronic SNPs in *CYP3A4* has been informed by the functional implications of C20493T (rs35599367; *CYP3A4*22*) in intron 6 [24]. This SNP results in the formation of a non-functional alternative splice variant in the liver [65] which significantly influences the pharmacokinetics of statins [24], immunosuppressants, midazolam, and erythromycin [66]. The intronic SNPs might play an indirect role in CYP3A4 adaptation in regioselectivity of oxidation of a wide range of diverse compounds metabolized by CYP3A4 [67,68]. We thus argue for further interrogation of the functional effects of previously identified and novel intronic variants.

In conclusion, we described the genetic variation in *CYP3A4* in Lake Victoria Kenyan and ni-Vanuatu populations living in malaria-endemic regions of East Africa and the Pacific, respectively. The contrasting frequencies of *CYP3A4* SNPs identified in these populations, including the novel C4614T SNP in a ni-Vanuatu population, highlight the need for pharmacogenetic investigations in other ethnic groups to potentially aid and inform region-specific malaria treatment guidelines, given the significance of CYP3A4 as an antimalarial drug-metabolizing enzyme. Thus, we urge for continued generation of human genetic data and the development of the necessary infrastructure for pharmacogenetic research.

CRediT authorship contribution statement

K.M, C.W.C, E.M.G.R, P.O, M.N, W.K, M.H, J.G, A.K formulated and designed experiments, K.B.M, performed experiments, data analysis, manuscript writing, C.W.C, P.O performed experiments, data analysis, C.W.C, E.M.G.R, P.O, C.K, T.O, J.K, M.N, W.K, B.K M.H, Y.K, J.G, A.K interpretation of data, manuscript review and final manuscript approval.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We want to thank the study participants for their consent to use their samples and the support of the fieldwork staff at the study sites. This work (PI: AK & J.G) was financially supported by a Grant-in-Aid from the Japan Agency for Medical Research and Development (AMED)/ Japan International Cooperation Agency (JICA) joint research project, under the joint Science and Technology Research Partnership for Sustainable Development (SATREPS), grant number 20JM0110020H0002, the Japan Society for the Promotion of Science (JSPS) Grants-in-Aid for Scientific Research (KAKENHI), grant number 22K0129, Hitachi Ltd Hitachi Fund Support for Research Related to Infectious Diseases, the Swedish Research Council (Vetenskapsrådet), grant numbers 2022-04055 and 2022-0363, and Formas, grant number 2022-02360. K.M. was supported by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dmpk.2024.101029.

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