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Molecular investigation of the *Pfmdr*1 gene of *Plasmodium falciparum* isolates in Henan Province imported from Africa

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Abstract

Efficacious antimalarial drugs are important for malaria control and elimination, and continuous monitoring of their efficacy is essential. The prevalence and distribution of *Pfmdr*1 were evaluated in African migrant workers in Henan Province. Among 632 isolates, 13 haplotypes were identified, NYSND (39.87%, 252/632), YYSND (2.85%, 18/632), NFSND (31.01%, 196/632), NYSNY (0.47%, 3/632), YFSND (13.77%, 87/632), NFSNY (0.32%, 2/632), YYSNY (2.06%, 13/632), YFSNY (0.16%, 1/632), N/Y YSND (1.90%, 12/632), N Y/F SND (6.17%, 39/632), N/Y Y/F SND (0.47%, 3/632), YYSN D/Y (0.16%, 1/632) and N/Y FSND (0.79%, 5/632). The highest frequency of NYSND was observed in individuals from North Africa (63.64%, 7/11), followed by South Africa (61.33%, 111/181), Central Africa (33.33%, 56/168), West Africa (28.94%, 68/235) and East Africa (27.03%, 10/37) ($\chi^2 = 54.605$, $P < 0.05$). The highest frequency of NFSND was observed in East Africa (48.65%, 18/37), followed by West Africa (39.14%, 92/235), Central Africa (26.79%, 45/168), South Africa (22.65%, 41/181) and North Africa (9.09%, 1/11) ($\chi^2 = 22.368$ $P < 0.05$). The mutant prevalence of codons 86 and 184 decreased. These data may provide complementary information on antimalarial resistance that may be utilized in the development of a treatment regimen for Henan Province.

Introduction

Malaria is a parasitic disease that has historically threatened human health, particularly in tropical and sub-tropical regions (Zhou *et al.*, 2016). It is considered as one of the three major global public health problems, together with HIV/AIDS and tuberculosis (Vitoria *et al.*, 2009). In early 2016, malaria was reported to be endemic in 91 countries and territories, and about 212 million cases and 429 000 deaths occurred globally in 2015 (WHO, 2016), of which more than 90% were in Africa. In 2010, the government of China announced its plan to eradicate malaria in the entire country by the end of 2020. The action plan was thus launched that same year in Henan Province. With the progress of malaria elimination, no local malaria cases have been reported in Henan Province since 2012 (Liu *et al.*, 2014*a*). However, with increasing travel, migration, economic globalization and international exchanges, malaria, especially *Plasmodium falciparum*, has re-emerged as a major public health challenge (Feng *et al.*, 2014; Liu *et al.*, 2014*b*), with over 90% of cases originating from Africa (Liu *et al.*, 2014*c*; Zhang *et al.*, 2015; Yang *et al.*, 2016).

A total of 212 million malaria cases were reported in 2015, reflecting a 14% decline since 2010 and 22% since 2000. The observed decrease in malaria prevalence was mainly attributable to the application of efficacious antimalarial drugs. Among these, chloroquine (CQ) has been proven to be one of the most effective drugs against malaria, particularly in the highly endemic regions of Africa (Wellems and Plowe, 2001). The success of CQ and its extensive use have led to the development of resistance in *P. falciparum* from the late 1950s (Maberti, 1960; Moore and Lanier, 1961; Reyes, 1981; Peters, 1987). CQ resistance was first reported in 1957 at the Thailand–Cambodia border, which eventually spread to other countries around the world (Wernsdorfer and Payne, 1991; Mehlotra *et al.*, 2001; Ridley, 2002). Artemisinin combination therapies (ACTs) were then recommended against uncomplicated *P. falciparum* infections in 2006 (WHO, 2006). ACTs were considered to be the most effective treatment for *P. falciparum*. However, in 2008, artemisinin-resistance strains were reported in Western Cambodia, followed by cases in Southeast Asia and the Great Mekong Subregion (GMS) (Muller *et al.*, 2009; Wang *et al.*, 2015).

*Pfmdr*1, a gene located on *P. falciparum* chromosome 5, encodes P-glycoprotein homologue-1, which is a molecular marker for a variety of antimalarial drugs. Mutations involving codons 86, 184, 1034, 1042 and 1246 have been shown to be strongly linked to a variety of antimalarial drug resistances, including artemisinin (Guan *et al.*, 2005; Gama *et al.*, 2010). In Africa, resistance to CQ, amodiaquine (AQ) and antifolate antimalarial drugs has been observed (Trape *et al.*, 2002; Roper *et al.*, 2003), and a decreased response to artesunate (AS) plus AQ has also been reported (Nsobya *et al.*, 2007). Although there was no evidence of artemisinin resistance in Africa, its impending spread was predicted to

Table 1. PCR primer sequences used for the amplification sequence encoding *pfmdr*1

Gene	Primer	Sequence (5′–3′)	Size (bp)
*Pfmdr*1 86, 184	mdr-1-F	TTA AAT GTT TAC CTG CAC AAC ATA GAA AAT T	612
	mdr-1-R	CTC CAC AAT AAC TTG CAA CAG TTC TTA	
	mdr-2-F	TGT ATG TGCTGT ATT ATC AG GA	526
	mdr-2-R	CTC TTC TAT AAT GGA CAT GGT A	
1034, 1042, 1246	mdr-3-F	AAT TTG ATA GAA AAA GCTATT GAT TAT AA	880
	mdr-3-R	TAT TTG GTA ATGATT CGA TAA ATT CAT C	
	mdr-4-F	GAA TTA TTG TAA ATG CAG CTT TA	799
	mdr-4-R	GCA GCA AAC TTA CTA ACA CG	

Table 2. The polymorphisms of *pfmdr*1 gene

Gene	SNP	Occurrence of mutation		
		N	%	95% CI
*Pfmdr*1 (*n* = 380)	N86Y	141	22.31	20.65–25.55
	Y184F	341	53.96	50.07–57.85
	S1034C	0	0	0
	N1042D	0	0	0
	D1246Y	21	3.32	1.92–4.72

SNP, single-nucleotide polymorphism; CI, confidence interval.

be disastrous (Mok *et al.*, 2011). In Henan Province, all registered malaria cases since 2012 were imported, with over 90% involving migrant workers from Africa. It is thus essential to understand the distribution of antimalarial drug resistance to assist in the development of prevention, control and elimination schemes for malaria in Henan Province. Therefore, this study was designed to evaluate drug resistance marker polymorphisms in migrant workers in Henan Province to provide rational suggestions to prevent and treat future cases. In this study, the prevalence of polymorphisms in the *Pfmdr*1 gene was determined from *P. falciparum*-positive African patients in Henan Province from 2012 to 2016.

Materials and methods

Sample collection and DNA extraction

Blood samples were collected from *P. falciparum*-infected African migrant workers prior to treatment and were labelled with study numbers, names and dates. All patients returned from Africa to Henan Province in 2012–2016. The patients were finally diagnosed by microscopic examination of Giemsa-stained thick and thin blood smears and nested polymerase chain reaction (PCR). All the blood samples were stored at −20 °C until analysis. DNA was isolated from the blood samples using the QIAamp DNA Mini kit (QIAGEN Inc., Frankfurt, Germany) according to the manufacturer's instructions and stored at −20 °C until PCR analysis.

Pfmdr1 gene amplification and sequencing

Amino acid substitutions N86Y, Y184F, S1034C, N1042D and D1246Y in the *Pfmdr*1 gene were screened. Polymorphisms were evaluated using nested PCR. The primary round of PCRs was performed in a total volume of 25 μL, which consisted of the following: 8.5 μL of ddH$_2$O, 1 μL each of first-round primer (10 μmol L^{-1}), 12.5 μL of a 2 × Go Taq Green Master Mix (PROMEGA Inc., Madison, WI, USA) and 2 μL of the DNA

template. The PCR reaction conditions were as follows: 95 °C for 3 min, followed by 30 cycles of 95 °C for 30 s, 54 °C for 45 s and 72 °C for 30 s; and a final extension of 72 °C for 5 min. The second round contained the second-round primers and 1.5 μL of the first-round PCR product as template, with the same cycling conditions as that described for the first round. The primer sequences were shown in Table 1. Sequencing was conducted by Shanghai DNA BioTechnologies Co., Ltd. (Shanghai, China).

Sequencing alignments and data analysis

Sequences were analysed using BLAST (http://blast.ncbi.nlm.nih. gov/) and aligned to reference sequence PF3D7_0523000 using BioEdit Sequence Alignment Editor. The data were analysed using Microsoft Excel and SPSS 17.0. *Pfmdr*1 allele frequencies were calculated with Microsoft Excel and differences were assessed using SPSS 17.0. Pearson's χ^2 test was used to determine the significance of the results. *P* values were calculated and considered to be statistically significant at $P < 0.05$.

Results

Epidemiological characteristics of patients

A total of 632 isolates were collected from returning *P. falciparum*-infected African migrant workers in Henan Province in 2012–2016. The male:female ratio was 125.4:1 (627/5). The mean age was 38.09 ± 9.34 years (range: 17–70), of which only five patients were older than 60 years and one was <18 years of age. The 632 patients returned from 29 countries in Africa, with the majority (37.18%, 235/632) returning from West Africa, followed by South Africa (28.64%, 181/632) and Central Africa (26.58%, 168/632). Only a minority of the patients came back from East Africa and North Africa, accounting for 5.86% (37/632) and 1.74% (11/632) of the total number of patients, respectively (Table 2).

Screening for Pfmdr1 mutations and genotyping

The amplicon of locus 86 and 184 was 526 bp in length, and the amplicon including locus 1034, 1042 and 1246 was 799 bp in length. The amplicons were successfully sequenced from all samples. Positions 1034 and 1042 of the *Pfmdr*1 gene were all wild-type. Mutations involving codons 86, 184 and 1246 of the *Pfmdr*1 gene were identified in 380 patients. The mutation Y184F was the predominant change (53.96%) (Table 2), and 13 haplotypes were identified, including wild-type haplotype NYSND (39.87%, 252/632), three single-mutant haplotypes YYSND (2.85%, 18/632), NFSND (31.01%, 196/632) and NYSNY (0.47%, 3/632), three double-mutant haplotypes YFSND (13.77%, 87/632), NFSNY (0.32%, 2/632) and YYSNY

Table 3. Distribution of *Pfmdr*1 polymorphisms from Africa-imported cases

Region	Country	Total	Wild type NYSND	Single mutant type			Double mutant type			Triple mutant type YFSNY	Mixed type				
				YYSND	NFSND	NYSNY	YFSND	NFSNY	YYSNY		N/Y YSND	N Y/F SND	N/Y Y/F SND	YYSN D/Y	N/Y FSND
North Africa		11	7 (63.64%)	0	0	0	1 (9.09%)	0	3 (27.27%)	0	0	0	0	0	0
	North Sudan	7	6 (85.71%)	0	0	0	1 (20.00%)	0	0	0	0	0	0	0	0
	Libya	4	1 (25.00%)	0	0	0	0	0	3 (75.00%)	0	0	0	0	0	0
South Africa		181	111 (61.33%)	15 (8.29%)	41 (22.65%)	1 (0.55%)	3 (1.66%)	1 (0.55%)	0	0	1 (0.55%)	7 (3.87%)	0	0	1 (0.55%)
	Mozambique	14	8 (57.14%)	0	5 (35.72%)	0	1 (7.14%)	0	0	0	0	0	0	0	0
	Zambia	21	14 (66.67%)	2 (9.52%)	4 (19.05%)	0	0	0	0	0	0	1 (4.76%)	0	0	0
	Angola	145	88 (60.69%)	13 (8.97%)	32 (22.07%)	1 (0.69%)	2 (1.38%)	1 (0.69%)	0	0	1 (0.69%)	6 (4.14%)	0	0	1 (0.69%)
	Zimbabwe	1	1 (100.00%)	0	0	0	0	0	0	0	0	0	0	0	0
East Africa		37	10 (27.03%)	0	18 (48.65%)	1(2.70%)	1 (2.70%)	1 (2.70%)	0	0	1 (2.70%)	4 (10.81%)	0	0	1 (2.70%)
	South Sudan	3	1 (33.33%)	0	1 (33.33%)	0	1 (33.34%)	0	0	0	0	0	0	0	0
	Ethiopia	5	0	0	5 (100.00%)	0	0	0	0	0	0	0	0	0	0
	Kenya	4	2 (50.00%)	0	0	0	0	0	0	0	1 (25.00%)	1 (25.00%)	0	0	0
	Uganda	13	3 (23.08%)	0	6 (46.16%)	1 (7.69%)	0	1 (7.69%)	0	0	0	2 (15.38%)	0	0	0
	Tanzania	12	4 (33.33%)	0	6 (50.00%)	0	0	0	0	0	0	1 (8.33%)	0	0	1 (8.33%)
West Africa		235	68 (28.94%)	1 (0.43%)	92 (39.14%)	0	44 (18.72%)	0	5 (2.13%)	1 (0.43%)	5 (2.13%)	17 (7.23%)	0	1 (0.43%)	1 (0.43%)
	Burkina Faso	1	0	0	1 (100.00%)	0	0	0	0	0	0	0	0	0	0
	Mali	4	1 (25.00%)	0	1 (25.00%)	0	1 (25.00%)	0	0	0	0	1 (25.00%)	0	0	0
	Togo	5	1 (20.00%)	1 (20.00%)	2 (40.00%)	0	1 (20.00%)	0	0	0	0	0	0	0	0
	Benin	6	2 (33.33%)	0	3 (50.00%)	0	1 (16.67%)	0	0	0	0	0	0	0	0
	Ivory Coast	10	3 (30.00%)	0	6 (60.00%)	0	1 (10.00%)	0	0	0	0	0	0	0	0
	Liberia	22	3 (13.64%)	0	3 (13.64%)	0	8 (36.36%)	0	2 (9.09%)	0	1 (4.55%)	4 (19.05%)	0	1 (4.55%)	0
	Ghana	33	12 (36.36%)	0	15 (45.46%)	0	3 (9.09%)	0	0 (0.00%)	0	0	3 (9.09%)	0	0	0
	Sierra Leone	32	10 (31.25%)	0	10 (31.25%)	0	4 (12.50%)	0	2 (6.25%)	1(3.12%)	2 (6.25%)	3 (9.38%)	0	0	0
	Guinea	33	6 (18.18%)	0	14 (42.42%)	0	8 (24.25%)	0	0	0	1 (3.03%)	3 (9.09%)	0	0	1 (3.03%)
	Nigeria	87	28 (32.18%)	0	37 (42.53%)	0	17 (19.54%)	0	1 (1.15%)	0	1 (1.15%)	3 (3.45%)	0	0	0
	Senegal	2	2 (100.00%)	0	0	0	0	0	0	0	0	0	0	0	0

(*Continued*)

Table 3. *(Continued.)*

Region	Country	Total	Wild type NYSND	Single mutant type			Double mutant type			Triple mutant type YFSNY	Mixed type				
				YYSND	NFSND	NYSNY	YFSND	NFSNY	YYSNY		N/Y YSND	N Y/F SND	N/Y Y/F SND	YYSN D/Y	N/Y FSND
Central Africa		168	56 (33.33%)	2 (1.19%)	45 (26.79%)	1 (0.59%)	38 (22.62%)	0	5 (2.98%)	0	5 (2.98%)	11 (6.55%)	3 (1.79%)	0	2 (1.19%)
	Central African Republic	4	2 (50.00%)	0	1 (25.00%)	0	0	0	0	0	1 (25.00%)	0	0	0	0
	Chad	6	3 (50.00%)	0	2 (33.33%)	0	1 (16.67%)	0	0	0	0	0	0	0	0
	Gabon	6	2 (33.33%)	0	2 (33.33%)	0	1 (16.67%)	0	0	0	0	0	0	0	1 (16.67%)
	Congo, DRC	16	9 (56.25%)	0	3 (18.75%)	0	2 (12.50%)	0	1 (6.25%)	0	0	0	1 (6.25%)	0	0
	Congo	28	13 (46.44%)	2 (7.14%)	7 (25.00%)	0	2 (7.14%)	0	2 (7.14%)	0	0	1 (3.57%)	1 (3.57%)	0	0
	Cameroon	27	6 (22.22%)	0	9 (33.33%)	0	6 (22.22%)	0	2 (7.41%)	0	0	4 (14.81%)	0	0	0
	Equatorial Guinea	81	21 (25.93%)	0	21 (25.93%)	1 (1.23%)	26 (32.10%)	0	0	0	4 (4.94%)	6 (7.41%)	1 (1.23%)	0	1 (1.23%)
Total		632	252 (39.87%)	18 (2.85%)	196 (31.01%)	3 (0.47%)	87 (13.77%)	2 (0.32%)	13 (2.06%)	1 (0.16%)	12 (1.90%)	39 (6.17%)	3 (0.47%)	1 (0.16%)	5 (0.79%)

(2.06%, 13/632), one triple-mutant haplotype YFSNY (0.16%, 1/632) and five mixed haplotypes N/Y YSND (1.90%, 12/632), N Y/F SND (6.17%, 39/632), N/Y Y/F SND (0.47%, 3/632), YYSN D/Y (0.16%, 1/632) and N/Y FSND (0.79%, 5/632). NFSND was the predominant haplotype (51.58%, 196/380, Table 3 and Fig. 1). The sequences of the mutations were deposited in GenBank under accession number MH266472-MH266479.

Distribution of the Pfmdr1 haplotypes

Thirteen haplotypes encompassing codons 86, 184, 1034, 1042 and 1246 of the *Pfmdr*1 gene were identified in this study. Only one patient who returned from Sierra Leone in West Africa harboured the YFSNY haplotype. The wild-type haplotype NYSND was detected in patients originating from various regions of Africa. The other haplotypes were found in patients from two, three or four regions of Africa. Eleven isolates from patients who returned from two countries in North Africa carried three haplotypes: NYSND (72.73%, 8/11), YFSND (9.09%, 1/11) and YYSNY (27.27%, 3/11). Approximately 181 samples from patients originating from four countries in South Africa carried the wild-type haplotype NYSND (61.33%, 111/181), three single-mutant haplotypes YYSND (8.29%, 15/181), NFSND (22.65%, 41/181) and NYSNY (0.55%, 1/181), two double-mutant haplotypes YFSND (1.66%, 3/181) and NFSNY (0.55%, 1/181), and three mixed haplotypes N/Y YSND (0.55%, 1/181), N Y/F SND (3.87%, 7/181) and N/Y FSND (0.55%, 1/181). Around 37 patients from five countries in East Africa carried eight haplotypes, including the wild-type haplotype NYSND (27.03%, 10/37), two single-mutant haplotypes NFSND (48.65%, 18/37) and NYSNY (2.70%, 1/37), two double-mutant haplotypes YFSND (2.70%, 1/37) and NFSNY (2.70%, 1/37), and three mixed haplotypes N/Y YSND (2.70%, 1/37), N Y/F SND (10.81%, 4/37) and N/ Y FSND (2.70%, 1/37). Approximately 235 isolates from patients originating from 11 countries in West Africa carried 10 haplotypes, including the wild-type haplotype NYSND (28.94%, 68/235), two single-mutant haplotypes YYSND (0.43%, 1/235) and NFSND (39.14%, 92/235), two double-mutant haplotypes YFSND (18.72%, 44/235) and YYSNY (2.13%, 5/235), one triple-mutant haplotype YFSNY (0.43%, 1/235), and four mixed haplotypes N/Y YSND (2.13%, 5/235), N Y/F SND (7.23%, 17/235), YYSN D/Y (0.43%, 1/235) and N/Y FSND (0.43%, 1/235). Approximately 168 isolates from patients from seven countries in Central Africa carried 11 haplotypes, including the wild-type haplotype NYSND (33.33%, 56/168), three single-mutant haplotypes YYSND (1.19%, 2/168), NFSND (26.79%, 45/168) and NYSNY (0.59% 1/168), two double-mutant haplotypes YFSND (22.62%, 38/168) and YYSNY (2.98%, 5/168), and five mixed haplotypes N/Y YSND (2.98%, 5/168), N Y/F SND (6.55%, 11/168), N/Y Y/F SND (1.79%, 3/168), YYSN D/Y (0.59%, 1/168) and N/Y FSND (1.19%, 2/168). The highest frequency of the wild-type haplotype NYSND was observed in patients from North Africa (63.64%, 7/11), followed by South Africa (61.33%, 111/181), Central Africa (33.33%, 56/168), West Africa (28.94%, 68/235) and East Africa (27.03%, 10/37). Significant differences among groups were observed ($\chi^2 = 54.605$, $P < 0.05$). NFSND was the predominant mutant haplotype in patients from East Africa, accounting for 48.65% (18/37) of the total number of haplotypes, followed by those from West Africa (39.14%, 92/235), Central Africa (26.79%, 45/168), South Africa (22.65%, 41/181) and North Africa (9.09%, 1/11). Significant differences among groups were observed ($\chi^2 = 22.368$, $P < 0.05$).

Distribution of Pfmdr1 mutations based on year

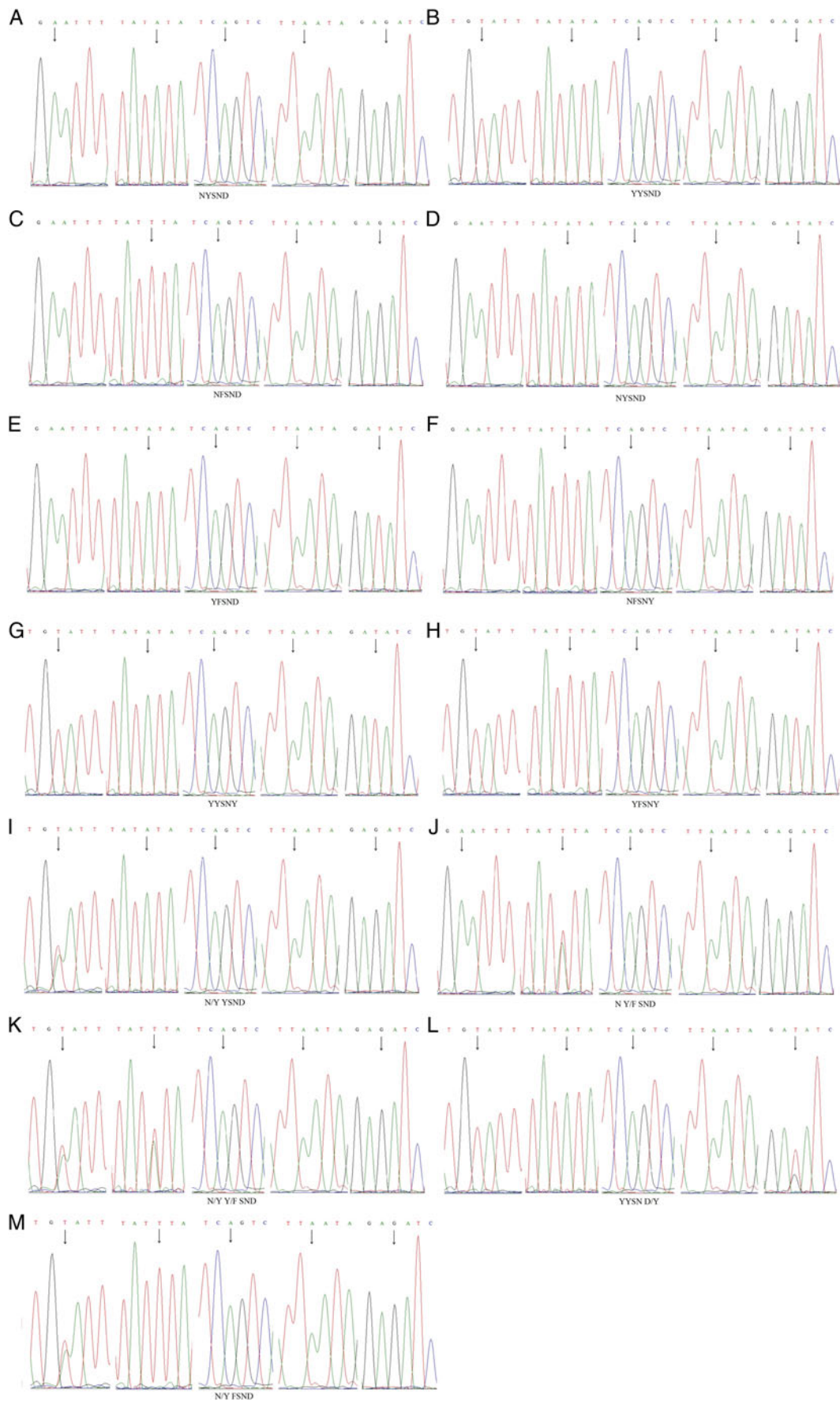From 2012 to 2016, the frequency of mutations involving codon 86 was 34.44% (31/90), 29.46% (38/129), 22.54% (32/142),

Fig. 1. Codons 86, 184, 1034, 1042 and 1246 haplotypes in the *pfmdr*1 gene of *P. falciparum* samples from Africa migrant workers in Henan Province. (A) The wild-type haplotype. The haplotype NYSND of sample 3D7. (B)–(H) The mutant haplotypes. The haplotypes were named YYSND, NFSND, NYSNY, YFSND, NFSNY, YYSNY and YFSNY, respectively. (I)–(M) The mixed haplotypes. The haplotypes were named N/Y YSND, N Y/F SND, N/Y Y/F SND, YYSN D/Y and N/Y FSND. Mutation are indicated by arrows.

Table 4. The mutations of *Pfmdr*1 reported in 2012–2016

Year	No. cases	No. *Pfmdr*1 genetic mutations		
		Locus 86	Locus 184	Locus 1246
2012	90	31	58	2
2013	129	38	70	5
2014	142	32	73	7
2015	131	27	79	3
2016	140	13	61	4
Total	632	141	341	21

20.61% (27/131) and 9.29% (13/140), respectively, and differences among frequencies were statistically significant ($\chi^2 = 25.372$, $P < 0.05$). From 2012 to 2016, the frequency of mutations involving codon 184 was 64.44% (58/90), 54.26% (70/129), 51.41% (73/142), 60.31% (79/131) and 43.57% (61/140), respectively, with significant differences among the years ($\chi^2 = 12.564$, $P < 0.05$). The frequency of mutations involving codon 1246 was 2.22% (2/90), 3.88% (5/129), 4.93% (7/142), 2.29% (3/131) and 2.86% (4/140), respectively, with no significant differences among years ($\chi^2 = 2.133$, $P > 0.05$) (Table 4).

Discussion

Drug resistance in *P. falciparum* is a global problem that has severely affected malaria control. To design an effective scheme for malaria control, it is essential to understand the patterns and distribution of antimalarial drug resistance. Although molecular markers do not directly reflect drug resistance in the malaria parasite, these may be utilized in early surveillance of alleles that undergo selection pressure. Molecular markers are currently the most convenient and efficient methods in resistance monitoring.

The volume of trade and services between China and other countries has increased in recent years. Malaria cases in Henan Province involving migrant workers have thus markedly increased, although no local cases of malaria have been reported in Henan Province since 2012. Many of the malaria cases are imported cases of *P. falciparum*, most of which are from Africa. The management of imported cases has become the main task for malaria control in Henan Province. The use of molecular markers for drug-resistance monitoring will provide data that may be used for the management of imported malaria cases.

The *Pfmdr*1 gene has been proposed as a molecular marker for CQ resistance, and it has also been reported to be associated with *in vitro* responses to AS and AQ (Nsobya *et al.*, 2007; Danquah *et al.*, 2010; Lin *et al.*, 2010). In our study, *Pfmdr*1 polymorphisms were identified in imported *P. falciparum* isolates collected during 2012–2016 from migrant workers returning to Henan Province from Africa. The majority of the isolates (77.69, 100, 100, and 96.68%) carried wild-type N86, S1034, N1042 and D1246 at codons 86, 1034, 1042 and 1246, respectively, which agreed with that previously reported in isolates from the China–Myanmar border (Huang *et al.*, 2012) and Africa (Thomas *et al.*, 2002; Feng *et al.*, 2015). The high frequency of the *Pfmdr*1 N86Y allele in Africa has been attributed to the extensive use of AS plus AQ (Dokomajilar *et al.*, 2006; Froberg *et al.*, 2012). The percentage of samples carrying the mutation 184F at codon 184 was 53.96%, which was similar to the findings in Osogbo, Nigeria (Ojurongbe *et al.*, 2007).

The distribution of *Pfmdr*1 polymorphisms is related to geographic locations (Xu *et al.*, 2016). N86Y has been mainly

reported in Asia and Africa, whereas N1042D and D1246Y occur more frequently in South America (Thomas *et al.*, 2002; Lucchi *et al.*, 2015). The results of this study coincided with the lower frequency of N1042D and D1246Y mutations in Africa. Thirteen genotypes were detected in our study. The wild-type NYSND was the predominant haplotype (39.87%), followed by NFSND (31.01%) and YFSND (13.77%), whereas the other haplotypes showed low frequencies. There was the largest proportion of the mutant type in West Africa, accounting for 60.85% (143/235) (YYSND, 1/235, 0.43%; NFSND, 92, 39.14%; YFSND, 44, 18.72%; YYSNY, 5/235, 2.13%; and YFSNY, 1/235, 0.43%), this difference might be due to the amount of AQ used in different regions. In this study, 9.49% of the mixed-type (wild-type and mutant) *Pfmdr*1 was found in *falciparum* malaria isolates from patients who returned from 17 countries (Zambia, Angola, Kenya, Uganda, Tanzania, Mali, Liberia, Ghana, Sierra Leone, Nigeria, Guinea, Central African Republic, Gabon, Congo, DRC, Congo, Cameroon and Equatorial Guinea). This result confirmed the widespread distribution of *Pfmdr*1 in Africa. The mixed haplotype of *Pfmdr*1 has been reported in Angola, Sudan, Nigeria, Ghana and Mozambique (Plucinski *et al.*, 2015; Xu *et al.*, 2016). This study enriched the distribution of the mixed type of *Pfmdr*1. Among 632 isolates, only one patient, who returned from Sierra Leone, harboured the YFSNY haplotype.

The mutant type of *Pfmdr*1 was shown to be associated with resistance to lumefantrine *in vitro* and *in vivo* studies (Sisowath *et al.*, 2007; Mwai *et al.*, 2009; Conrad *et al.*, 2014; Henriques *et al.*, 2014). Mutations in *Pfmdr*1 were also considered to be associated with an increase in resistance to AS; therefore, it is valuable to monitor the resistance in various regions (Van Tyne *et al.*, 2013). Henan Province implemented an action plan for malaria elimination in 2010, and no local malaria cases have been reported since 2012; over 90% of the current cases involve migrant workers returning from Africa. Angola, Nigeria and Equatorial Guinea became the top three countries from which the patients returned (Yang *et al.*, 2016; Zhou *et al.*, 2016). In this study, 632 patients returned from 29 countries in Africa, and half of them returned from Angola (145), Nigeria (87) and Equatorial Guinea (81). Surveillance and population studies are essential for the early detection and subsequent prevention of the spread of drug resistance (Mvumbi *et al.*, 2015), and it is very important for the goal of eliminating malaria in Henan Province.

There was a trend of increasing prevalence of isolates with wild-type genotypes at these codons, including 86 and 184, from the year 2012 to 2016. It suggested that the resistance of *P. falciparum* to CQ might be reduced, and sensitivity might again be achieved. In this study, the prevalence of the combination of both mutations (86Y + 1246Y) was 2.06% in the parasite population. Based on this relatively low prevalence, currently, the use of ACTs as a first-line treatment is effective to treat *P. falciparum* in Henan Province. These data should help provide a more comprehensive picture of antimalarial resistance and guide decisions regarding treatment policies in Henan Province.

Conclusions

This study reported the prevalence of polymorphisms in the *Pfmdr*1 gene in returning African migrant workers in Henan Province. The frequencies of *Pfmdr*1 N86Y and Y184F alleles in the samples from Africa were relatively high, whereas the mutant frequencies of S1034C, N1042D and D1246Y were low. The frequency of isolates with mutations at codons 86 and 184 decreased from 2012 to 2016. It was essential to assess the evolution of antimalarial drug resistance in returning migrant workers in Henan Province, and these data will be useful in the development of an effective treatment policy in Henan Province.

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